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The Effect of Long-Term High-Carbohydrate Low-Fat Intake on Substrate Utilization and Plasma Lipoprotein Concentrations in Postmenopausal Women

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The Effect of Long-Term High-Carbohydrate Low-Fat Intake on Substrate Utilization and Plasma Lipoprotein Concentrations in Postmenopausal Women.

The objective of this study was to determine if changes in plasma lipoprotein concentrations that occur in response to high-carbohydrate, low-fat (HCLF) intake may be associated with or result from changes in substrate utilization. By implementing three diets which replaced energy from fat with carbohydrate, stepwise, over a 16-week period, we assessed the metabolic effects of a controlled increase in carbohydrate consumption in postmenopausal women. Substrate utilization was assessed by analysis of plasma metabolites [e.g. lactate, free glycerol (FG), and free fatty acids (FFA)] as well as respiratory quotient (RQ) measurements at baseline and three different timepoints throughout the study. Fasting insulin and glucose concentrations were measured to assess insulin sensitivity. Following the increase in carbohydrate consumption, mean fasting insulin concentration decreased nearly 24% and was accompanied by a concurrent drop in fasting glucose levels. Fasting RQ measurements, plasma FFA and FG concentrations following intervention did not significantly shift from baseline concentrations. Mean total-, low-density lipoprotein- and high-density lipoprotein-cholesterol were reduced an average of 5%, 9% and 13.5% of baseline values, respectively. Both plasma triglyceride (TG) and lactate were markedly elevated relative to baseline. Plasma lactate and TG correlated significantly at two of four timepoints, with plasma TG concentrations higher in those with higher plasma lactate levels. Our results suggest that during increased carbohydrate consumption, glucose oxidation increases, resulting in increased concentrations of plasma lactate. Thus, HCLF intake may promote lipogenesis by enhancing the levels of lipogenic precursors, such as lactate. The increase in lipogenesis induced through HCLF intake by increased lipogenic precursors causes the endogenous synthesis of FFA which in turn increases plasma TG. Additional research is warranted to more clearly define the relationship of increased production of lactate, a lipogenic substrate, to hypertriglyceridemia and increased plasma TG and cardiovascular disease risk in postmenopausal women.

REVIEW OF THE LITERATURE

INTRODUCTION

Increased blood cholesterol has been identified as a primary indicator of coronary heart disease (CHD) risk by national advisory boards whose mission is to prevent and reduce the incidence of cardiovascular disease (CVD) (1-3). Both the National Cholesterol Education Program (NCEP) and the American Heart Association (AHA) have identified low-density lipoprotein-cholesterol (LDL-C), the lipoprotein which transports mainly cholesterol, as the primary target of cholesterol-lowering therapy (2,3). Guidelines issued by the AHA and the NCEP Expert Panel direct health care providers to utilize dietary therapy as the fist line treatment of high blood cholesterol. Thus, the primary emphasis of current nutritional therapy to reduce plasma cholesterol concentrations and coronary risk is to lower LDL-C concentrations (2, 3).

Restriction of total and saturated fat intake is the cornerstone of dietary therapy aimed at lowering LDL-C concentrations (1-6). Therefore, guidance to reduce the risk of CVD risk has been accompanied with encouragement to increase carbohydrate consumption.

The average carbohydrate intake in America is currently 45% of an individual's total energy intake (7). Those with hyperlipidemia are frequently encouraged to increase carbohydrate consumption to as much as 65% of total energy intake (1, 2, 4-6). Consequently, adherence to these recommendations often necessitate remarkable modification of diet and perhaps lifestyle.

It is becoming increasingly clear that increased dietary carbohydrate consumption may induce undesirable outcomes including hypertriglyceridemia (HTG) and reduced high-density lipoprotein-cholesterol (HDL-C) (8-12). In addition, both HTG and low HDL-C concentrations have been shown to indicate increased CVD risk in women (13-15). Unfortunately, the metabolic cause(s) behind these changes subsequent to increased high carbohydrate consumption are not completely understood.

Researchers have speculated that the consumption of excess carbohydrate may promote the formation of fatty acids via de novo lipogenesis, ultimately leading to the increase of plasma TG observed subsequent to high-carbohydrate low-fat (HCLF) intake (16, 17) (**Appendix A**). Interestingly, a number of short-term HCLF studies have shown that lactate concentrations are increased following HCLF consumption (18-21). Moreover, a substantial body of evidence suggests that lactate, a 3-carbon intermediate of glucose metabolism, plays an important role as both a precursor and stimulator of lipogenesis *in vivo* (22, 23). These studies reveal that the primary products of lipogenesis, saturated fatty acids, are found in triglycerides (TG) and phospholipid (23). Other studies which investigated the effect of HCLF intake on carbohydrate utilization have shown that de novo lipogenesis does not appear to provide a significant contribution to the body's fat stores (24-27). The production of fatty acids stored as plasma TG instead of body fat may account for the disparity between energy balance studies which have discounted the possibility that increased carbohydrate consumption enhances de novo lipogenesis and induces HTG.

The following literature review provides an overview of the effect HCLF intake has on substrate utilization, presented in four sections. In **Section 1**, the role of lipogenesis in long-term energy balance is explored in detail. The effects of HCLF intake on systemic measurements of lipolysis and substrate oxidation are presented in **Section 2**. In **Section 3,** the influence FFA concentration has on glucose oxidation and storage is discussed. Lastly, the effects of HCLF intake on plasma lipoproteins are introduced in **Section 4**.

SECTION 1

Effects of HCLF Intake on Substrate Utilization

Long- & short-term energy balance

The tendency of individuals to maintain a stable body weight during long periods of their life reflects an ability to adapt to constant variations in nutrient intake (28, 29). Energy balance, the difference between energy intake and energy expenditure, is known to vary considerably on a day-to-day basis in free-living individuals.

Starch, sugars, and triglycerides provide most of the dietary energy consumed while glucose and free fatty acids (FFA) are the major metabolic fuels used for energy production. Long-term net energy balance is likely to be sustained only if the fuel mix oxidized by the body matches the relative contributions made to that mix by these nutrients from the diet.

Insulin's role in maintaining energy balance

The availability of glucose and amino acids to cells for nutrient utilization is as important to maintaining energy balance as is nutrient intake itself. The availability of glucose and amino acids to cells is largely dependent on effective insulin-mediated delivery. Insulin stimulates its target organs to store and conserve energy fuels while decreasing their rate of fuel oxidation. Primary sites of insulin action include adipose tissue, skeletal muscle, and the liver. Insulin activates the transport of glucose from the plasma into adipose tissue and muscle by mobilizing glucose transporters from the

cytoplasm to the cell membrane. These glucose transporters are actually vesicles which contain membrane-bound protein capable of transporting glucose into the cell (30). In contrast, glucose transport into the liver, brain, and red blood cells is not insulin-mediated (31).

In adipose tissue, insulin increases glucose transport and lipoprotein lipase (LPL) action while inhibiting lipolysis. By enhancing the facilitated diffusion of glucose from the plasma into adipose tissue, insulin promotes fatty acid synthesis. The transport of glucose into the cell increases the availability of both pyruvate for fatty acid synthesis and glycerol 3-phosphate for esterification of the newly formed fatty acids. By stimulating the activation of LPL in adipose tissue, insulin facilitates the uptake of TG fatty acids from circulating TG-rich lipoproteins, chylomicrons, and very-low-density lipoprotein (VLDL) into adipose tissue (32). In this way, insulin promotes fat storage as well as fat synthesis. Lastly, insulin reduces the concentration of plasma FFA through its inhibitory effect on hormone sensitive lipase (HSL). Hormone sensitive lipase, located within adipose tissue, catalyzes the hydrolysis of TG to FFA and free glycerol (FG) when insulin concentration is low (30).

Insulin increases glucose transport and oxidation, as well as glycogen synthesis and protein synthesis in skeletal muscle. Likewise, insulin inhibits protein catabolism and LPL activity in skeletal muscle. By enhancing the facilitated diffusion of glucose from the plasma into muscle, insulin promotes glycogen synthesis as well as protein synthesis. This action antagonizes that of glucagon which increases glycogenolysis and gluconeogenesis to increase the amount of glucose in the blood (33).

In the liver, insulin inhibits glycogen breakdown, gluconeogenesis, and VLDL secretion (32). Additionally, insulin promotes hepatic protein synthesis (30).

Insulin Insensitivity: it's effect on substrate utilization

Insulin resistance or insensitivity occurs when increased plasma glucose is present despite a normal insulin level or when a normal plasma glucose level is accompanied by elevated plasma insulin (34). Hyperinsulinemia is a hallmark of insulin resistance (35), which presumably develops to sustain the tissue-specific effects of insulin. Meanwhile, the body attempts to compensate for the compromised glucose delivery by deaminating amino acids and using the non-nitrogenous portion to synthesize glucose or fatty acids. Glucose accumulates in the blood since it is unable to cross cell membranes within skeletal or adipose tissue. The inhibited transport of glucose into insulin-dependent tissues ultimately results in hyperglycemia. Excess glucose is then excreted in the urine. As a result, negative energy balance ensues (30).

During insulin resistance, fatty acid oxidation in skeletal muscle is increased in an effort to adapt to decreased glucose delivery and the consequent reduction in glucose oxidation. Elevated lipolysis of adipose tissue and increased circulating FFA contribute further to the increased oxidation of fatty acids (36). In addition, an overproduction of VLDL triglycerides (37) and tissue-specific alterations in LPL result (38). A higher plasma level of insulin increases the activity of LPL and simultaneously decreases the activity of HSL (38). Thus, the net biochemical outcome is a greater percent of fat put into storage and proportionately less fat removed from storage for metabolism.

The effects of HCLF intake on insulin sensitivity

Diet is considered an important environmental factor which influences insulin-mediated glucose uptake. Although the degree to which dietary composition influences insulin sensitivity is not certain, one basis for the current recommendation for individuals to increase their carbohydrate intake is the theory that a high content of complex carbohydrates may lead to an improvement in insulin sensitivity (4, 39).

Himsworth was the first to propose that HCLF diets enhance insulin sensitivity sixty years ago (40). He demonstrated that the glucose tolerance of normal subjects increased as the carbohydrate content of the antecedent diet was progressively increased.

Studies by Brunzell et al.(41) and Anderson (42) have also suggested that when dietary carbohydrate content is increased to 75-85% of total calories, insulin sensitivity is enhanced. When these diets were implemented for 7-10 days, glucose tolerance was improved while plasma insulin response remain unchanged in both normal subjects (41) and patients with mild non-insulin dependent diabetes mellitus (NIDDM) (41, 42). Both authors conclude that high carbohydrate intake increases the sensitivity of peripheral tissues to insulin.

More direct evidence of the ability of high carbohydrate feedings to improve insulin sensitivity has been provided by Kolterman and associates (43). Healthy subjects were given a formula feeding, containing 75% carbohydrate. After consuming the formula for five days, subjects experienced mild postprandial hyperglycemia and more marked postprandial hyperinsulinemia while basal values were unchanged. Following fourteen days on the formula, subjects showed a similar response, except that the

elevations of plasma glucose and insulin concentrations were not as marked. To illustrate, the areas under both the insulin and glucose curves were significantly increased in the 5-day group, while only insulin concentrations were increased in the 14-day group.

Insulin sensitivity was estimated using a euglycemic clamp technique which allows the subject's ability to dispose of a measured glucose load under variable insulin stimulus to be quantified. To do this, an intravenous infusion of insulin and glucose is given while epinephrine and propranolol are simultaneously administered in an effort to suppress endogenous insulin secretion. A steady state plasma concentration of glucose and insulin is achieved within ninety minutes of the infusion. Thus, the mean steady state plasma glucose concentration is a direct reflection of the efficiency of insulin-mediated glucose uptake (43). Inversely, during an insulin clamp technique, insulin levels are progressively increased while the plasma glucose concentration is maintained at a predetermined level by the simultaneous infusion of glucose. In this way, researchers are able to measure the effect different insulin levels have on glucose uptake (44).

As indicated by the steady state plasma glucose, the high carbohydrate diet led to a marked enhancement of insulin's ability to augment glucose disposal which was similar after both the short-term and long-term feeding periods. Based on findings in rat studies, the authors suggest that the enhanced insulin sensitivity may be due to increased activity of the glucose transport system. Additionally, intracellular steps in glucose metabolism may be enhanced and contribute to insulin's ability to promote glucose removal.

Hjollund and associates (45) implemented the use of HCLF mixed meals to confirm findings previously reported following HCLF formula feedings. After NIDDM

subjects consumed a low-fat/high-starch/high-fiber diet for three weeks, diabetes control improved as demonstrated by decreased fasting plasma glucose and 24-hour urinary glucose excretion. Despite the improved glucose homeostasis, no significant changes were observed in fasting serum insulin levels. During an intravenous insulin tolerance test (IVITT), blood samples were drawn at eight timepoints following the intravenous injection of insulin. The rate of glucose disappearance was then quantified to determine the in vivo insulin action. The *in vivo* insulin action as estimated by the IVITT improved significantly in subjects consuming the alternative diet. Furthermore, in fat cells obtained from patients in the alternative-diet group, insulin receptor binding increased subsequent to the change of diet. For these reasons, the authors conclude the enhanced insulin action may be due, in part, to increased insulin receptor binding.

High carbohydrate-low fat intake is typically accompanied by generous fiber consumption when solid, mixed meals are consumed (46). A number of studies have related positive changes in fasting plasma glucose and insulin levels to increases in both carbohydrate and fiber intake (47-50). Their conclusions have been strengthened by a recent study examining the effect of a high-carbohydrate (68%), high-fiber diet. Fukagawa and colleagues reported the sensitivity of peripheral tissues to physiological concentrations of insulin was significantly increased in healthy adults after consuming a HCLF high fiber diet for 21 days or more (51). Following the HCLF high fiber period, fasting glucose and insulin were decreased by 5% and 24% respectively. Insulin-mediated glucose disposal was measured using the euglycemic clamp technique before and after the HCLF high fiber period. Glucose disposal rate was determined by the

glucose infusion rate necessary to maintain euglycemia. Mean glucose disposal increased by 143% of basal following 21-28 days on the modified diet. This effect was reversed in less than two weeks after normal dietary intake was resumed. These findings imply that an HCLF high fiber intake increases the sensitivity of peripheral tissues to physiological concentrations of insulin in healthy adults.

Insulin resistance and diminished β-cell sensitivity to glucose are characteristics which have been associated with aging in humans (52). Recognizing that elderly subjects reportedly consume less carbohydrate than their younger counterparts, Chen and colleagues proposed that dietary intake may be related to the decline in glucose intolerance observed in aging (52). To examine the relationship between dietary intake and insulin sensitivity in aging, a group of young (18-36 y) and elderly (65-82 y) nonobese healthy men were recruited. Frequently sampled intravenous glucose tolerance tests were performed, from which, the glucose disappearance rate, insulin sensitivity index, and β-cell responsiveness to glucose were derived. Although dietary carbohydrate intake during the ad libitum dietary period was similar in the young (41% carbohydrate/CHO) and older (49% CHO) groups, the older men were less glucose tolerant than the younger men. Glucose intolerance in the older men was related to both insulin resistance and decreased β-cell responsiveness. After consuming a very high carbohydrate (85% CHO/0% fat) formula feeding for three to five days, glucose tolerance was markedly improved in the older group. In fact, glucose tolerance in the older subjects became identical to that of the young subjects whose glucose tolerance was unchanged. The authors conclude that factors independent of an individual's carbohydrate intake

contribute to the decline in glucose tolerance associated with aging. First, carbohydrate's ability to enhance insulin sensitivity is reduced with aging. Secondly, reduced islet function also contributes to reduced glucose tolerance and insulin resistance associated with aging. The precise mechanisms by which increased carbohydrate intake improves pancreatic function and insulin sensitivity are poorly understood. However, this study demonstrates that differences in insulin sensitivity, islet function and carbohydrate tolerance between young and aged nonobese men are much less when carbohydrate intake is high.

Despite the above findings, the effect HCLF intake has on insulin sensitivity remains controversial. Analysis of Himsworth's data (53) indicates that most of the improvement in glucose tolerance and the associated index of insulin insensitivity occurred when carbohydrate intake was increased from less than 10% to approximately 30% of energy intake. Increases from 30 to 60% of energy intake, which are representative of the range of carbohydrate intake in western diets, produced little improvement. Furthermore, a sequence effect may have affected the results since subjects consumed each diet consecutively beginning with the lowest and ending at the highest carbohydrate content (40).

The above studies assessed the effect that antecedent diets, composed of varied amounts of carbohydrate, have on glucose and insulin responses. To do this, a variety of techniques were utilized, including oral and intravenous glucose tolerance tests, the euglycemic clamp technique, and an intravenous insulin tolerance test. By contrast, actual insulin and glucose response to variations in carbohydrate intake have been

reported in an effort to describe the consequences of replacing fat intake with carbohydrate (11, 12, 54). Ginsberg (12) and Reaven (11) each utilized liquid formula feedings to study the effects of moderate dietary fat restriction (55% CHO/30% fat) on glucose and insulin response. Both studies reported that the high carbohydrate formula led to significant increases in post-prandial plasma glucose and insulin levels. While Reaven and Olefsky observed significantly lower glucose and insulin levels three hours after the high carbohydrate formula feeding, Ginsberg et al. did not.

Coulston and colleagues incorporated two levels of dietary carbohydrate (40% and 60%) into typical U.S. diets (54). Fasting blood samples were drawn after each diet was fed for ten days to healthy volunteers. No changes were observed in fasting plasma insulin or glucose concentrations. While the mean plasma postprandial glucose levels were higher on the HCLF diet at each time period, none of these differences reached statistical significance. However, significant elevations in postprandial insulin response were observed before commencing the meal, as well as one and two hours following ingestion of the HCLF meal. Taken alone, these three studies may indicate that routine HCLF intake may induce day long plasma glucose and insulin levels (11). Still others maintain that circulating levels of glucose and insulin may not always appropriately reflect the current physiologic state (34).

Several studies reporting improved insulin sensitivity used extremely carbohydrate-rich diets (70-85% CHO). With more modest increases in carbohydrate intake, variable effects have been reported on the glucose profile in diabetic (55, 56) and healthy subjects (54, 57, 58). To illustrate, in a recent, well-publicized multi-center

study, NIDDM patients were given a high-carbohydrate diet containing 55% of the total energy as carbohydrates and 30% as fats. A high-monounsaturated-fat diet containing 40% carbohydrates and 45% fats was also included (56). Compared with the high-monounsaturated-fat diet, the high-carbohydrate diet increased daylong plasma glucose by 12% while concurrently increasing insulin values by 9%. These effects on plasma glucose and insulin levels persisted during the 14 week trial.

Fiber's impact on improving insulin sensitivity has also been questioned. Riccardi et al. (55) attempted to determine the separate effects of the fiber and carbohydrate components of the diet on the control of glucose metabolism in both type I (insulin-dependent) and type II diabetics. These investigators suggested that an increase of digestible carbohydrate without an increase in dietary fiber does not improve control of blood glucose concentrations. Once again however, the diet which contained the greatest proportion of carbohydrate (53% CHO) was substantially lower than those which previously demonstrated improved insulin sensitivity (68-85% CHO).

Therefore, it appears that very-high-carbohydrate, low-fat diets do promote improved glucose control and insulin sensitivity. Additionally, the inherent increase in fiber content of HCLF diets may also play a role in improved glucose homeostasis. Nonetheless, the effects that a more modest increase in carbohydrate intake has on insulin sensitivity and glucose tolerance is more variable.

Lipogenesis--its role in long-term energy balance

Unlike protein, dietary carbohydrate, as glucose, can be converted not only to glycogen, but also to fat (59). Conversion of excess carbohydrates into fat provides a pathway for the storage of carbohydrates consumed in excess.

Although the liver and adipose tissue contain the enzymes necessary for the conversion of carbohydrate to fat, it is fairly well established that lipogenesis in the adult human occurs primarily in the liver (60). This knowledge has led to the common perception that conversion of carbohydrate to fat is an important pathway for the retention of dietary energy and for the accumulation of body fat. Support for this concept gained support following observation of humans receiving intravenous hyperalimentation which contained concentrated dextrose solutions (16). As indicated by respiratory quotient (RQ) measurements greater than one, lipogenesis was occurring in these patients. Additionally, widespread observations of HTG in patients adhering to HCLF diets have led to speculation that high carbohydrate intake promotes de novo lipogenesis (17).

Saturated glycogen stores precede de novo lipogenesis

Acheson et al. (24) and other researchers (61, 62) have demonstrated that a considerable surplus of carbohydrate is necessary to induce measurable lipogenesis in humans. Acheson demonstrated that even after subjects consumed a meal which provided approximately 500 grams of carbohydrate, fat synthesis did not exceed the concomitant rate of fat oxidation. Indirect calorimetry was utilized to evaluate changes in the body's carbohydrate and fat content. The mean RQ value remained below 1.0 for

ten hours following meal consumption, indicating that there was not a significant net gain in body fat. These findings reflect those reported by Passmore and Swindells (61) over thirty years ago under more rudimentary experimental conditions. Acheson and colleagues were able to establish that only 1-2% of the 500-g carbohydrate meal was routed towards lipogenesis, while 85% was converted into glycogen stores. These findings substantiate that the previously established capacity for glycogen storage in humans, 300 grams, underestimates actual glycogen capacity. To their credit, Passmore and Swindells theorized this twenty years earlier (61).

HCLF intake promotes glycogen storage & glucose oxidation

Some sugars, including maize and cane sugar, have a detectable amount of ^{13}C-content which is naturally occurring. Naturally occurring sources of the stable isotope of carbon, ^{13}C, provide a cost effective source of ^{13}C-carbohydrate for studies in man. The use of ^{13}C has become increasingly popular for human metabolic studies due to its low health risk in comparison with ^{14}C. Additionally, improved mass spectrometer technology, used to detect stable isotopes, has made stable isotope studies more practical. For these reasons, Acheson and colleagues utilized a naturally labeled ^{13}C-carbohydrate load along with respiratory exchange measurements, to investigate exogenous glucose utilization (26).

Three groups of subjects consumed one of the following three antecedent diets: high in fat, high in carbohydrate, or mixed. After consuming the antecedent diet for three to six days, 500 g of carbohydrate, naturally labeled with ^{13}C, were ingested and respiratory exchange measurements were performed (26). In the high fat group, fat was

the principal energetic substrate, allowing much of the ingested carbohydrate to be stored as glycogen. Exogenous carbohydrate oxidation was greater in both the high carbohydrate and mixed diet groups than in the high fat group. These findings suggest that when the body glycogen stores are low, glucose uptake is facilitated in order to replete them. Conversely, a diet rich in carbohydrates will simultaneously maintain glycogen stores closer to their saturation levels while providing a greater proportion of carbohydrate for oxidation. Authors conclude that de novo lipogenesis can occur to a very limited extent, but only after both glycogen storage and glucose oxidation have become saturated (25, 26).

Carbohydrate oxidation is sensitive to the composition of dietary intake

Subsequent studies have established that carbohydrate balances are maintained more accurately than fat balances (63-65). To measure the impact fat intake has on fat oxidation, subjects consumed a mixed maintenance diet plus an additional amount of 106 ± 6g fat per day for 36 hours (64). Despite the addition of approximately one thousand calories per day of dietary fat, there was no appreciable change in the 24-hour energy expenditure or RQ. These findings confirm an earlier report (66) that dietary fat intake does not promote fat oxidation during the postprandial hours. Therefore, the regulation of fat balance differs markedly from the regulation of the carbohydrate balance because only carbohydrate balance is facilitated by a spontaneous adjustment of the rate of oxidation to intake. As a result, substantial imbalances between intake and oxidation are much more likely for fat than for carbohydrate (64).

J.P. Flatt (65) confirmed that carbohydrate balance continues to be maintained more accurately than fat balance even in the long-term. Mice were maintained on one of two diets for 160 days: a high carbohydrate diet (69% CHO, 13% fat) or a "mixed western diet" (37% CHO, 45% fat). Daily variations in the amounts of food consumed were found to exert opposite effects on carbohydrate and fat oxidation. Thus, carbohydrate oxidation was found to be correlated positively with daily food, and hence carbohydrate intake, whereas fat oxidation was correlated negatively with daily food and thus fat intake. Fat oxidation appeared to be determined by the gap between overall energy expenditure and the sum of carbohydrate plus protein energy ingested, rather than by the amount of fat consumed.

Possible limitations of indirect calorimetry

It may be argued that conclusions based on the above studies are limited by the fact that indirect calorimetry is a measurement of net lipogenesis. In other words, fatty acid synthesis could be masked by concomitant fatty acid oxidation, or futile cycling. However, the energy cost of transforming glucose to lipid is far greater (23% glucose energy) than when the same amount of glucose is converted to glycogen (7%) (25, 67). Consequently, a noticeably increased energy expenditure would signal the presence of futile cycling. Nonetheless, in an effort to measure lipogenesis directly, Hellerstein and colleagues (27) have successfully employed a stable-isotopic method for measuring de novo lipogenesis in humans. Their findings are consistent with previous conclusions

based on indirect calorimetry. Thus, de novo lipogenesis appears to be a quantitatively minor pathway and fatty acid futile cycling is not thermogenically significant.

Specific dietary factors may enhance de novo lipogenesis

Hudgins et al. attempted to determine the amount of carbohydrate intake which would favor lipogenesis in humans (68). To do this, subjects were provided formula diets of varying amounts of carbohydrates. The fatty acid composition in the drinks was designed to reflect the fatty acid composition of adipose tissue. Typically, plasma VLDL triglycerides contain fat derived from either adipose tissue or from the diet. However, when the fatty acids consumed are comparable to those in VLDL, newly-formed VLDL triglycerides composition should reflect the fatty acid composition of adipose tissue. However, if de novo synthesis of fat from carbohydrate ensues, a decline in linoleic acid (an essential fatty acid) content of VLDL would indicate the presence of de novo lipogenesis. De novo lipogenesis was detected only when subjects consumed a formula diet containing 10% of the energy as fat energy. These findings were confirmed by Hudgins and colleagues by using [^{13}C] acetate incorporation into VLDL triglyceride palmitate (68).

The technique described above was utilized to determine distinct factors that may enhance de novo lipogenesis (69). Lean volunteers were fed two high carbohydrate diets for 25 days each. Both the liquid formula diet and the solid food diet contained 75% carbohydrate and 10% fat. Additionally, the fatty acid composition was matched to each subject's adipose tissue composition. The carbohydrate content of the formula feeding

was largely derived from glucose polymers. In contrast, the solid food diet was higher in complex carbohydrates (50% starch) and fiber (~40g/d). The dilution of 18:2 in VLDL TG relative to the percent 18:2 in the adipose tissue indicated that nearly half of VLDL TG fatty acid may have been formed de novo in the volunteers fed the eucaloric very low fat fiber-free diet with glucose polymers. Conversely, de novo lipogenesis was suppressed by the low-fat, solid food diet containing fiber and more complex carbohydrates.

Previous findings on the effect of propionate on fatty acid synthesis may elucidate these findngs. The short-chain fatty acid, propionate, is produced by colonic microflora upon fermentation of dietary fiber. Using isolated rat hepatocytes, Nishina and Freedland demonstrated that lipogenesis was effectively inhibited by the addition of proprionate to cell preparations (70).

The above findings indicate that the source of carbohydrate, along with the amount of fiber intake, may have a significant impact on the likelihood of de novo lipogenesis occurring. Despite the dramatic differences in the diet-induced de novo lipogenesis, energy expenditure measured by indirect calorimetry did not differ between diets.

Summary

The consumption of carbohydrate promotes glucose storage and oxidation (71). Lipogenesis from carbohydrate appears to be limited by the body's preference to store excess glucose as glycogen. Carbohydrate intake promptly increases carbohydrate oxidation and continues to do so once glycogen reserves are repleted (25, 66). As a result, HCLF diets spare fat from oxidation, even if fat is consumed along with the carbohydrate (66).

Conversely, lower glycogen levels are maintained on diets which are proportionately lower in carbohydrate and higher in fat content. Since the rate of fat oxidation is markedly influenced by the availability of carbohydrate, this provides a mechanism permitting increased oxidation of fat. The body's ability to increase or decrease the use of carbohydrate as metabolic fuel plays an important role in the maintenance of carbohydrate balance.

SECTION 2

Effects of HCLF intake on systemic measurements of
lipolysis & substrate oxidation

Free Glycerol (FG) & Free Fatty Acids (FFA)

During the resting state, or the interval between feeding and fasting, liver and

muscle derive most of their energy from fatty acids which are released from adipose

tissue into the bloodstream. As metabolism transitions from the fed to the fasting

condition, glucose availability from food decreases and liver glycogen is utilized to

maintain the blood glucose. In the fully fasted state, endogenous glucose production

(from amino acids and glycerol) does not keep pace with its utilization and oxidation.

As a result of diminishing glucose availability, the inhibitory effect of insulin on lipolysis

abates, which induces the activation of HSL. Upon activation, HSL catalyzes the

hydrolysis of TG to FFA and FG (30).

Once mobilized, FFA, bound to albumin, are transported to the tissues to be

either oxidized or esterified. The rate of fat oxidation generally varies in parallel with

changes in the level of circulating FFA (36, 72). Consequently, as the concentration of

FFA increases during fasting, FFA are oxidized at an ever-increasing rate in an effort to

spare the non-obligatory oxidation of glucose. Those FFA not oxidized are converted to

TG, packaged into VLDL and sent back to the bloodstream (30).

Free glycerol released during lipolysis can be used for the synthesis of TG or

oxidized within the adipose tissue. Additionally, the provision of glycerol for

gluconeogenesis also serves a vital function (73). Glycerol may join the carbohydrate

pool after activation to glycerol 3-phosphate which occurs primarily in the liver and kidney. However, muscle lacks the enzyme glycerokinase, which is required for introduction of glycerol into the glycolysis pathway. As a result, glycerol that is liberated from TG by LPL cannot be used directly by the muscle (31).

The effect long-term (i.e. >1 week) HCLF intake has on fasting or post-prandial FFA has not been reported to my knowledge. However, fasting and post-prandial FFA and FG have been measured as indicators of lipolysis and reesterification in a few substrate oxidation studies which utilized short-term implementation of HCLF diets.

In two separate studies (25, 26), Acheson reported antecedent diets of variable macronutrient content had pronounced effects on fasting plasma FFA levels in healthy males. Fasting FFA levels were significantly greater in subjects who had received a high fat (14% CHO:75% fat) than in the subjects who received either mixed (61% CHO:27% fat) or high carbohydrate (80% CHO:9% fat) diets. Additionally, following a 500 g carbohydrate load, plasma FFA concentrations in the high fat group dropped, but stabilized at a higher level than in the mixed or low fat groups. Likewise, an additional collection of studies indicates that postprandial concentrations of FG and FFA typically reach lower postprandial levels following meals with the higher carbohydrate to fat ratio (66, 74, 75).

However, Leclerc and colleagues (58) reported that antecedent diets of variable carbohydrate content had no effect on FFA concentration following a 75g glucose load. While these results appear to conflict with the above findings, this study design did not include a diet containing greater than 30% fat content. Therefore, differences in FFA

values may have been more difficult to detect due to less variation in diet composition. Additionally, the 75g glucose challenge provided only a fraction of the carbohydrate or glucose that was provided in the above measurements. These factors may have affected study outcome.

Van Amelsvoort and co-workers (74, 75) illustrated that postprandial plasma FFA and FG concentrations display similar patterns of change following the consumption of variable amounts of carbohydrate and fat. After an initial rapid drop shortly after the start of the meals, FFA and FG concentrations show a rise after the fat-rich meals. Conversely, FFA and FG decline further following carbohydrate-rich meals.

Rationale for the observed decrease in FFA levels which appears to be concurrent with HCLF intake may be three-fold. Reduced lipolysis is an anticipated outcome in response to the elevated insulin levels induced by increased carbohydrate intake. Secondly, both the high level of glucose (which is converted to glycerophosphate) and the concomitantly high level of insulin in the blood stimulate an increase in the rate of de novo synthesis of triacylglycerol from FFA. Lastly, release of FFA from plasma triacylglycerol is reduced following HCLF intake due to the limited postprandial rise in triacylglycerol. Inversely, the rise in FFA which followed the high fat meal is apparently due to the release of FFA by the action of LPL on triacylglycerols in the chylomicrons (74, 75).

Lactate

Lactate, a product of glycolysis and glycogenolysis, is produced by erythrocytes and skeletal muscle (76). According to the classical pathway of lactate production, lactate is produced primarily in tissues that function under hypoxic circumstances (77). To illustrate, glycolysis in red blood cells always terminates in lactate, even in aerobic conditions. Complete oxidation of glucose involves conversion to pyruvate, followed by entry into the mitochondrion for conversion to carbon dioxide. Because erythrocytes are lacking mitochondria which contain the enzymatic machinery for the aerobic oxidation of pyruvate, glucose is oxidized only as far as pyruvate. The pyruvate is then reduced to lactic acid, which is released into the bloodstream to be processed further by the liver (78, 79).

Researchers agree that the absence of oxygen will cause an acceleration in glycolysis and increased lactate production. However, it has recently been proposed that the production or appearance of lactate may not always reflect a lack of oxygen (76, 80). It is postulated that the intracellular enzymes which process carbohydrates produce lactic acid as a function of metabolism.

According to the anaerobic threshold theory of lactate production, blood lactate concentration abruptly increases when the ventilary threshold is reached. Correspondingly, the production of lactate is a direct result of an absence of oxygen (78). On the contrary, there is evidence to support that significant amounts of lactate are formed (and removed) in healthy resting or exercising individuals who are not limited in terms of oxygen transport or utilization (76, 81). Brooks (80) contends that elevations

in blood lactate concentration are due to an inequality of lactate appearance (Ra) in the blood and its disappearance (Rd) from the blood. This mechanism has been coined the lactate shuttle.

It is well established that blood lactate and pyruvate concentrations increase after glucose administration (82, 83). In addition, elevations in plasma lactate concentration have been associated with HCLF intake (18-21). The impact HCLF intake has on postprandial plasma lactate was illustrated by Surina and colleagues. Following the consumption of just one HCLF meal (78% CHO:6% fat), postprandial plasma lactate levels were significantly increased; lactate levels remained elevated throughout the three-hour testing period. Conversely, plasma lactate levels were not affected by the consumption of a high fat (33% CHO:52% fat) meal. Using the hyperinsulinemic clamp technique during euglycemia and hyperglycemia, Felley (21) reported that variations of lactatemia paralleled those of nonoxidative glucose disposal, which was markedly increased by hyperglycemia. These results support the existence of a lactate shuttle and suggest that lactate may even be a marker of glucose storage.

The augmented formation of lactate versus pyruvate has been attributed to lactate dehydrogenase (LDH), the terminal enzyme of the glycolytic pathway. The fact that the catalytic activity of LDH (V_{max}) far exceeds the activities of alternative enzymes competing for pyruvate, may explain the diversion of glycolytic carbon flow into lactate. In addition to the activity of LDH, its thermodynamic equilibrium and k_m (0.08 mM) also favor the conversion of pyruvate to lactic acid (80). For these reasons,

Brooks and associates (80, 84) conclude that lactic acid will inevitably be formed as a result of accelerated metabolism.

The essence of the lactate shuttle has been confirmed in animals by Issekutz (85) who infused [^{3}H] glucose and [^{14}C]lactate into exercising dogs. In those animals, β blockade during exercise inhibited muscle glycogenolysis and caused the lactate appearance rate to decline and approach the glucose disappearance rate.

If the lactate shuttle does operate, one might consider why nature would provide for the formation of lactate under fully aerobic conditions. Interestingly, the formation of lactate appears to endow the organism with a great deal of flexibility in solving problems of metabolic regulation. As pointed out in tracer kinetic studies of lactate metabolism in humans, lactate may serve a vital role in distributing oxidizable substrate to the body (81, 76). Mazzeo and colleagues (81) reported that approximately 50% of lactate turnover is metabolized to carbon dioxide at rest. Furthermore, oxidation of lactate becomes the major fate of lactate removal during exercise. Alternatively, if lactate is not oxidized, it may be converted to glycogen or the lipid precursor, acetyl-CoA (76).

Lactate's role in the synthesis of glycogen has received much attention. It has long been accepted that after the ingestion of a carbohydrate load, a large fraction of the absorbed glucose is taken up by the liver and directly converted into glycogen (86). However, several laboratories have demonstrated that glucose, even in the presence of insulin, is a poor precursor for glycogen synthesis (86-89). It is now established that

only 40 to 50% of hepatic glycogen formed after an overnight fast, is from glucose after its phosphorlylation in the liver, or by the "direct" pathway" (90).

Conversely, the remainder of glycogen is generated by the gluconeogenic or "indirect" pathway (90, 91). To illustrate, following the administration of ~100g of glucose, gluconeogenic (indirect) production of glycogen in humans was found to be ~15g, compared with 10g of glucose which metabolized directly into glycogen (91) These findings have been strengthened by similar findings reported by Magnusson and co-workers (92) who established that the indirect pathway of liver glycogen formation is operative in the fed state as well as the fasted state. Researchers have reasoned that the limited capacity for hepatic glucose utilization may be due to a low level of glucokinase combined with the variable rates of futile cycling caused by the activity of glucose-6 phosphatase (86, 93).

For glycogen formation to proceed by the indirect pathway, glucose from dietary carbohydrate enters the systemic circulation and is transported to the periphery, where it is converted to lactate. Because of the large glycolytic capacity of skeletal muscle, it is assumed that muscle is the site of lactate formation. Lactate released into the systemic circulation is then taken up by the liver and converted to glucose 6-phosphate (via gluconeogenesis) and ultimately to glycogen (94).

While lactate may be serve as a gluconeogenic substrate, or be oxidized and utilized for energy, lactate may also function as a precursor for lipogenesis. Evidence in rodents demonstrates that glucose administration acts as a trigger for lipid synthesis, but the hexose serves only indirectly (via lactate or glycogen) as a precursor for lipogenesis

in the liver (22). In this study, rats refed a high carbohydrate diet after a fast exhibited a very high liver glycogen content and very efficient hepatic lipogenesis. Furthermore, hepatocytes from these rats showed that glycogen is the major source of lipid. Therefore, it appears that refeeding after a fast leads first to replenishment of the glycogen stores which then serve as a source of pyruvate and acetyl-coenzyme A for lipogenesis (93).

To summarize, lactate may undergo one of three possible metabolic fates. If not oxidized via the citric acid cycle, lactate may reenter the circulation as glucose. Alternatively, if glycogen stores are sufficient, lactate may be converted to acetyl-CoA in order to serve as a precursor for lipid synthesis. To my knowledge, the effect long-term HCLF consumption has on fasting plasma lactate levels has not been reported. Since it is well established that lactate can serve as a substrate for either gluconeogenesis or lipogenesis, this information may be very valuable.

Ketones

In the fed state, glycogen and fatty acid synthesis are active. Inversely, fatty acid oxidation and thus, ketone body production is virtually absent. The abundance of insulin in the fed state is offset by a low concentration of glucagon. Malonyl-CoA, the first committed intermediate in fatty acid biosynthesis, is present in maximal concentration and ultimately leads to long-chain fatty acid synthesis. Newly formed FFA are converted to TG, packaged as VLDL molecules, and transported to adipose tissue for storage (94).

Each one of these processes is reversed subsequent to only a few hours of fasting. Glycogen breakdown replaces glycogen synthesis, activating gluconeogenesis. The increase in glucagon which accompanies the fasting state as well as diabetes, provides the stimulus for hepatic ketogenesis to start (94). A decrease in malonyl-CoA concentration is coupled with a rise in hepatic carnitine, a cosubstrate essential for the ketogenic process (95). With the cessation of fatty acid synthesis, FFA are mobilized to the liver from peripheral fat stores. The rate at which FFA are oxidized increases while esterification to TG and phospholipids declines (94, 96, 97). The concomitant increase in hepatic carnitine levels and FFA ensure the onset of ketogenesis (94, 95).

Once activated, the rate of ketogenesis is determined primarily by the availability of FFA (98). In normal persons, excessive lipolysis is prevented through insulin release which is stimulated when ketone body levels reach 2-4 mM (94). In the absence of insulin, as in Type I diabetes, this safeguard is lacking. Therefore, when lipolysis and ketone body formation is uncontrolled, ketoacidosis is not prevented (94).

During ketogenesis, the liver produces acetoacetic and β-hydroxybutyrate (BHB), or ketone bodies. By enhancing its capacity to convert incoming fatty acids into ketone bodies, the liver provides auxiliary fuel sources for nonhepatic tissues. The body relies on ketones as an alternative fuel source when carbohydrate is in short supply or cannot be efficiently utilized. Additionally, ketogenesis spares glucose for certain tissues and cells such as the central nervous system and erythrocytes which are dependent on a continual supply of glucose. In prolonged starvation, ketones spare the breakdown of muscle protein for purposes of gluconeogenesis (98).

Since the ketone body, BHB, is formed almost exclusively in the liver secondary to fatty acid oxidation (18), it provides a convenient and measurable indicator of hepatic fatty acid oxidation. β-hydroxybutyrate concentrations were compared in men to investigate whether the fat content of a single meal influences fat oxidation (18). The plasma BHB level steadily increased with time following the high fat meal (33%CHO: 52% F) while it remained constant following the high carbohydrate (78% CHO: 6% F) meal. Furthermore, BHB measurements following the high fat condition were significantly higher at all timepoints than after the high carbohydrate meal. These results indicate that hepatic fatty oxidation was higher following the high fat meal as compared to the equicaloric high carbohydrate meal. Since meals were equicaloric and the carbohydrate content of the two test meals were not equal, these results do not appear to conflict with previous conclusions regarding substrate oxidation.

Indirect calorimetry & respiratory quotient (RQ)

The biochemical pathways by which the macronutrients, carbohydrate, fat and protein are metabolized in the body are well established. Despite this, factors which determine the flux through these pathways are less well understood. The use of RQ is one experimental approach which has been utilized to estimate the flux of major nutrients through different pathways.

The RQ compares the amount of inspired oxygen required to metabolize dietary intakes of individual nutrients with the amount of carbon dioxide expired (99, 100). Because the number of hydrogen atoms that must be combined with oxygen to form

water varies between metabolic fuels, each fuel is characterized by a different RQ. For example, since the number of CO_2 molecules produced equals the number of oxygen molecules consumed, the RQ for the complete oxidation of carbohydrate is 1.00. The number of hydrogen atoms that must be combined with oxygen to form water is much greater in fats than in either proteins or carbohydrates. Therefore, the oxidation of carbohydrate and protein is more efficient with respect to oxygen consumption than is fat and the resultant RQ for fat, 0.70, reflects this (101).

The RQ changes as the body shifts the types of body fuels being oxidized. The body's ability to change the rates of flux through different pathways permits the supply of appropriate nutrients to different tissues under different physiological conditions. To illustrate, fat provides the major source of energy during fasting; therefore, fasting RQs are typically ~0.7-0.8. In contrast, the RQ is very sensitive to carbohydrate intake. Accordingly, the RQ will rise to ~1 during a meal, regardless of the fat content in the meal (31). The length of time the RQ remains elevated is related to the carbohydrate content of the meal as well as the body's glycogen stores previous to the meal (18).

As illuded to, energy balance is also reflected in the RQ. For example, when energy intake remains below the amount of energy expended, the fuel mixture will contain a higher proportion of fat than what is provided in the diet. Hence, the ensuing RQ will approach 0.7 (102). Conversely, energy intake in excess of energy expenditure will result in an elevated RQ (103).

The noninvasive nature of indirect calorimetry makes it a convenient experimental approach to measure the effect substrate utilization has on energy

expenditure. By concurrently measuring a subject's rate of oxygen consumption and their carbon dioxide production over a given amount of time, the researcher can calculate the amount of energy expended. This estimation is based on the assumption that all the oxygen is used to oxidize degradable fuels and all the carbon dioxide produced is recovered . Moreover, indirect calorimetry provides information on the type and rate of substrate utilization in vivo (101).

Additionally, indirect calorimetry can be combined with other research methods. Stable isotope analysis allows the assessment of substrate oxidation through the measurement of plasma FFA oxidation. In contrast, indirect calorimetry estimates net whole body lipid oxidation. Therefore, the difference between these values may provide an estimate of tissue lipid oxidation (103).

SECTION 3

The Glucose Fatty Acid Cycle

Substrate utilization which is independent of hormonal control

Over twenty years ago, Randle and colleagues proposed the existence of a

glucose fatty acid cycle, by which enhanced release of FFA from muscle or adipose

tissue alters glucose metabolism in muscle independently of hormonal control (36).

Evidence is now available to verify that this cycle operates in both heart and skeletal

muscle in humans (104).

The mechanism by which FFA inhibit glucose metabolism remains controversial.

An increase in FFA oxidation by muscle will increase the molar ratios of NADH/NAD

and acetyl CoA/CoA (36). The increase in acetyl CoA can inhibit pyruvate

dehydrogenase activity and lead to augmented release of lactate and diminished glucose

oxidation (105). Realizing this, Randle et al. originally proposed that decreased rates of

glucose flux through the oxidative pathway would favor redirection of glucose

metabolism towards glycogen synthesis (36).

Other researchers (21, 106) have suggested that these changes in metabolic

milieu initiate a chain of metabolic events which affect glucose storage as well as

glucose oxidation. The accumulation of citrate is favored by the inhibition of the Krebs

cycle (by the increased NADH/NAD ratio) and the increase in acetyl CoA. Citrate, a

potent inhibitor of phosphofructokinase, slows down glycolytic flux. With the inhibition

of phosphofructokinase, glucose-6-phosphate accumulates and the glycolytic enzyme,

hexokinase, becomes inhibited. The proposed end result is decreased glucose transport into muscle cells with a consequent decrease in glycogen formation.

Insulin enhances glucose utilization

Insulin can alter the regulation of the glucose fatty acid cycle by virtue of its effect on glucose uptake. Once glucose is absorbed by tissues, glucose disposal may occur through either oxidative or nonoxidative mechanisms. The oxidation of glucose to carbon dioxide and water occurs by the citric acid cycle or the pentose shunt. Nonoxidative glucose disposal may result in glycogen synthesis, anaerobic glycolysis, conversion of glucose to fat, or the transformation of intermediary products of glucose into amino acids (107). Quantitatively, glycogen synthesis is the most important pathway for nonoxidative glucose disposal (21).

Additionally, insulin enhances glucose utilization by inhibiting FFA lipolysis (108) and enhancing FFA esterification, (109). Insulin also enhances glucose utilization through its action on FFA. The plasma FFA concentration is determined by the rate of FFA lipolysis occurring in adipose tissue and the rate of FFA disappearance from plasma. Removal of FFA occurs by one of two pathways: FFA may be oxidized to carbon dioxide and water or reesterified into TG (44).

Elevated plasma FFA levels inhibit insulin stimulated glucose utilization

Randle and colleagues (36) reported that metabolic changes observed following the administration of FFA to muscles of normal fed rats were also present in perfused hearts and diaphragms of alloxan diebetic rats. This observation led Randle et al. to propose that elevated plasma concentrations of FFA are responsible for the concomitant decrease in insulin sensitivity.

By infusing healthy subjects with a triglyceride emulsion, such as Intralipid, researchers are able to measure the effect elevated plasma FFA have on healthy individuals. The increases in FFA levels achieved by infusing lipid are similar to those observed in other insulin resistant conditions including obesity, diabetes, and disorders of lipid metabolism (106). However, the acute induction of elevated FFA through lipid infusion may not be an ideal model for insulin resistant conditions in that FFA are chronically elevated in obesity, diabetes, and disorders of lipid metabolism. The effect of chronically elevated FFA on FFA turnover and cellular glucose metabolism may be quantitatively different from the results obtained in acute studies (106). Nonetheless, the infusion of lipid emulsion provides a practical way to examine the impact FFA have on glucose metabolism.

Using lipid infusion, Ferrannini and colleagues examined the consequences of physiological elevations of FFA concentrations on glucose production and utilization by creating conditions of euglycemic hyperinsulinemia and hyperglycemic hyperinsulinemia in healthy volunteers (105). Their work illustrates that acute, physiological elevations of

plasma FFA levels inhibit insulin-stimulated glucose utilization and that hyperglycemia does not overcome this inhibition.

Groop and colleagues studied the mechanisms of insulin resistance in the presence of elevated plasma FFA. To examine FFA and glucose metabolism they used the sequential insulin clamp technique in combination with indirect calorimetry and infusion of $[3-^3H]$ glucose and $[1-^{14}C]$ palmitate (44). Because the presence of an enlarged fat mass, as seen in obesity, is associated with an increased supply of plasma FFA (72, 110, 111), obese subjects were selected for this study. The increased supply of plasma FFA which characterized the obese subjects appeared to result from increased lipolysis of the available fat mass. In obese subjects, higher insulin dosages were required for the stimulation of glucose disposal and inhibition of hepatic glucose production than in lean controls. Additionally, FFA metabolism was less sensitive to insulin in obese than in lean controls. However, the elevated FFA concentrations could be normalized at insulin concentrations in the high physiological range. These findings suggest that in uncomplicated obesity the enhanced FFA flux emerges from the enlarged fat mass but is under physiological control by (high) insulin concentrations (44). Additionally, the increased availability and oxidation of FFA leads, by the glucose fatty acid cycle, to the impairment of glucose utilization.

Groop and co-workers provided additional insights to the insulin insensitivity seen in human obesity by using a stepwise insulin clamp technique in combination with indirect calorimetry and infusion of $[1-^{14}C]$ palmitate (112). Once again, basal FFA turnover was enhanced and less suppressed by insulin in obese subjects. However, FFA

turnover expressed per kilogram fat mass was normal and normally suppressed by insulin. From this data, the authors conclude, in subjects with uncomplicated obesity, the adipose cell is normally sensitive to insulin and increased FFA turnover is simply a consequence of the enhanced fat mass.

Elevated plasma FFA levels decrease glucose disposal

Calorimetric studies have confirmed that increased lipid oxidation does result in the inhibition of glucose oxidation. Using two types of glucose tolerance tests along with indirect calorimetry, Gomez and colleagues (113) showed that infusion before a glucose load was followed by an increased lipid oxidation and that carbohydrate oxidation was diminished. The glucose load without fat infusion induced a rapid increase of carbohydrate oxidation and a decrease of lipid oxidation. Conversely, glucose tolerance was found to be depressed by the simultaneous administration of lipids and the insulin response to a glucose load was significantly increased. Findings were similar following a 90-minute i.v. glucose infusion with and without lipid infusion. The rate of disappearance of blood glucose was significantly decreased during lipid administration. A similar inhibition of glucose oxidation by FFA has been described following the ingestion of oral (114) or intravenous (105, 113, 115) glucose in man.

Despite the elevated insulin concentrations typically associated with excessive adipose tissue mass, increased FFA concentrations accompany increases in fat mass (72). The transport of FFA inside the cell for oxidation appears to be dependent upon the

plasma FFA concentration (44). As a result, FFA utilization may depend primarily on their plasma concentration. If plasma FFA concentration is the major determinant of the rate of plasma FFA oxidation, it seems likely that net lipid oxidation is increased in subjects who have an expanded fat mass.

To determine if fat oxidation is increased in those with increased fat mass, Golay and colleagues (72) studied the contribution of fat oxidation to energy utilization in obese subjects by using continuous indirect calorimetry in the course of an oral glucose tolerance test (OGTT). Fasting FFA levels were slightly but not significantly increased in the obese patients when compared to lean controls. However, basal lipid-oxidation as well as total lipid oxidation following the glucose load was increased in the obese subjects. Several other recent studies have also indicated that lipid oxidation may be enhanced in obese as well as diabetic subjects (116-118).

Elevated FFA limit insulin's ability to stimulate glucose oxidation

By employing a euglycemic insulin clamp technique in combination with indirect calorimetry, Thiebaud et al. (107) demonstrated that the ability of insulin to stimulate glucose oxidation is quite limited. During the infusion of insulin at five progressively increasing levels, glucose uptake sequentially rose with increasing plasma insulin concentrations. At the same time, glucose oxidation concurrently represented a progressively smaller amount of glucose uptake. When hyperglycemia was created, glucose oxidation failed to increase significantly above that observed during euglycemic

hyperinsulinemic conditions. Conversely, the total rate of glucose uptake increased nearly three-fold. These results indicate that insulin's ability to stimulate glucose oxidation is limited. Secondly, at high rates of glucose uptake, glucose storage may represent the major fate of glucose disposal.

Felber and colleagues (116) utilized both euglycemic insulin clamp and OGTT methods along with continuous indirect calorimetry to measure substrate oxidation and nonoxidative glucose disposal in obese subjects. As previously reported by Gomez (113) and others (44, 115), increased rates of lipid oxidation were observed in obese subjects in the postabsorptive state (OGTT), and in response to insulin stimulation (insulin clamp) in comparison with lean controls. Basal glucose oxidation was significantly decreased in obese subjects compared with age-matched lean controls. During both the OGTT and the euglycemic clamp, insulin-stimulated glucose oxidation was far lower in obese subjects than in lean subjects. These findings suggest that in obese subjects, characterized by increased rates of lipid oxidation, the stimulation of glucose oxidation by insulin is blunted. Interestingly, nonoxidative glucose disposal was markedly inhibited in obese patients during the euglycemic insulin clamp yet during the OGTT, glucose uptake was normal.

Elevated FFA inhibit both oxidative and non-oxidative glucose disposal

Utilizing the euglycemic insulin clamp technique in combination with indirect calorimetry, Thiebaud (106) demonstrated that FFA exert an inhibitory effect on glucose utilization through both glucose oxidation and glycogen synthesis. At each of three insulin dosages, hyperlipidemia caused a significant reduction in total glucose uptake which was reflected by a decrease in both total glucose oxidation and glucose storage. Additional subjects studied during a lower infusion rate of Intralipid revealed that maintaining FFA concentration constant at basal level is sufficient to inhibit the insulin-mediated glucose utilization.

Glucose storage values are obtained as the difference between total glucose disposal and glucose oxidation. During the lipid infusion, the decrease in glucose storage, or glycogen synthesis, during each of the three insulin clamp studies was greater than the decrease in glucose oxidation. These findings suggest the inhibitory effect of elevated FFA levels on glycogen synthesis contributed more to the impairment in glucose tolerance than did the defect in glucose oxidation.

Despite the findings reported by Thiebaud and colleagues (106), when a similar lipid infusion was given during an OGTT, hyperglycemia developed but no change in glucose storage was measured (119). As pointed out earlier, the results reported by Felber (116) showed that the inhibition of glucose storage secondary to elevations in plasma FFA only becomes evident during the euglycemic hyperinsulinemic clamp. This suggests that the impairment in glycogen synthesis observed exclusively during the

euglycemic hyperinsulinemic clamp is compensated for by a rise in glycemia and insulinemia during the OGTT. Additionally, physiological hyperglycemia along with hyperinsulinemia may function as a compensatory mechanism to offset the defect in nonoxidative glucose metabolism concomitant with elevated FFA oxidation (116).

Hyperglycemia--a regulatory mechanism for increased glucose storage?

For this reason, Felley and co-workers assessed the role of hyperglycemia on the lipid -induced inhibition of glucose storage during steady-state conditions of glycemia and insulinemia (21). Healthy volunteers were studied on four occasions using the hyperinsulinemic clamp technique, twice during euglycemia and twice during hyperglycemia, with and without Intralipid. Comparison of oxidative and nonoxidativve glucose disposal during euglycemia (90 mg/dl) and hyperglycemia (150 mg/dl) enabled Felley and co-workers to confirm that hyperglycemia concurrent with hyperinsulinemia is a potent stimulus of nonoxidative glucose disposal (107). During euglycemia, oxidative and nonoxidative glucose disposal occurred in equal proportions. By contrast, the amount of nonoxidative glucose disposal nearly doubled that of oxidative disposal during the 150 mg/dl hyperglycemia. These findings imply that glucose oxidation saturates under conditions in which glycogen synthesis can still increase (21).

Following lipid infusion, insulin-mediated glucose disposal was significantly impaired. However, the proportion of glucose disposed oxidatively or nonoxidatively was not affected. Thus, lipid infusion during euglycemia resulted in oxidative and non-

oxidative glucose diposal in a one-to one ratio. Conversely, lipid infusion during hyperglycemia caused the oxidative and nonoxidative disposal ratio to drop so that nonoxidative glucose disposal nearly doubled oxidative glucose disposal. This study confirms the existence of physiological regulatory mechanisms in which the rise in plasma FFA inhibits both glucose oxidation and glucose disposal or glycogen formation (106).

If the Intralipid infusion employed in the above study can represent a model for the increase in lipid metabolism observed in insulin resistant conditions, these results imply that the slight increase in glycemia that follows the inhibition of glucose disposal compensates for decreased glucose disposal as a positive-feedback mechanism. Additionally, the glucose intolerance frequently recognized in obesity and other insulin resistant conditions may serve as a response to decreased insulin-mediated glucose disposal while providing a regulatory mechanism by which glucose storage can still proceed, but at higher glycemian (21).

Unfortunately, the increased glucose storage induced by hyperglycemia and hyperinsulinemia may in turn heighten the insulin resistant condition. Because glucose storage is regulated, in part, by the metabolic state that precedes glucose administration (120), the compensatory rise in glycemia reduces the glucose mobilization from glycogen stores. As a result, glucose levels have less time to return to basal levels. Consequently, less glucose is being mobilized and the resistance to glucose becomes greater, inducing greater insulin insensitivity (120).

Summary

Elevated plasma FFA appear to inhibit glucose utilization through both oxidative and nonoxidative pathways (106). However, the capacity to increase glucose storage appears to exceed the body's limited capability to increase glucose oxidation during insulin resistant conditions (21). Hyperglycemia concurrent with hyperinsulinemia is a potent stimulus of nonoxidative glucose disposal (107). Therefore, it appears that Randle and colleagues (36) were partially correct in their assumption that glucose metabolism is redirected towards glycogen synthesis in response to decreased rates of glucose flux through the oxidative pathway. However, the stimulus for increasing nonoxidative glucose disposal appears to be hyperglycemia instead of FFA.

SECTION 4

The Effect of HCLF Intake on Plasma Lipoproteins

Intervention trials measuring the effect of HCLF intake on lipoproteins

Three dietary factors have been identified that raise serum LDL-C levels: these include saturated fatty acids, dietary cholesterol, and excess caloric intake leading to obesity (121). Both cholesterol and saturated fat affect plasma LDL-C levels by down-regulating the LDL receptor and inhibiting the removal of LDL-C from the plasma by the liver. Additionally, increased fat intake results in an increased flux of chylomicron remnants into the liver with a concomitant influx of cholesterol ester. Hence, the restriction of fat intake has become the cornerstone of dietary recommendations aimed at reducing the risk of CHD (3).

While several nutrients including polyunsaturated fatty acids, monounsaturated fatty acids and carbohydrates, can be substituted for saturated fatty acids to produce a reduction in LDL-C levels, this review will focus on the effects HCLF intake has on lipoprotein metabolism. Several lines of evidence have established that diets in which complex carbohydrate is substituted for saturated fat lead to decreased concentrations of plasma LDL-C. Both very high carbohydrate diets (>75% CHO) (122-124) and moderately high carbohydrate diets (50-65% CHO) (10, 125-127) have been demonstrated to reduce plasma total-cholesterol and LDL-C.

Because very high carbohydrate diets are difficult to achieve, most studies examining the effects of very high carbohydrate intake have utilized formula feedings to

provide the desired energy combination (122, 123). In contrast, Thuesen and colleagues (124) treated CHD patients with a diet containing 75% carbohydrate and 10% of total energy from total fat for three months. At the end of the third month, serum cholesterol had decreased 33% and serum LDL-C had decreased 41% from pretreatment values. The mean reduction of serum cholesterol was twice the amount predicted from the formula of Hegsted et al (128). The authors attribute this to an average weight loss of 8.6% occurring during this period. Serum TG and HDL-C concentrations were unchanged from the prediet period to the end of month three. After month three, subjects were asked to maintain a diet as low in fat as possible for long-term treatment. While average fat intake increased to 21.4%, a value still considered restrictive by most treatment guidelines, serum HDL-C values increased by 18% from baseline at the end of twelve months. Furthermore, serum TG decreased by 27% from baseline levels. Subjects were encouraged to replace fat calories with whole grain products and to limit sugar intake. This may explain, in part, the unexpected increase in serum HDL-C and decrease in serum TG at the twelve month point. Additionally, the reduction in body weight which occurred in this population most likely had a favorable effect on serum TG.

While most HCLF intervention studies have been limited to men, Geil (129), Kasim (125), and Denke (10) have measured the effect of HCLF intake on lipid metabolism in women. The total cholesterol and LDL-C-lowering-effects of HCLF diets in women have been demonstrated through study diets containing 50-65% energy from carbohydrate (10, 125, 129). Kasim and co-workers described the long-term effects of a diet providing 15% of total energy from fat on lipoprotein metabolism of healthy women.

Following one year of diet modification, total cholesterol was decreased 7% and LDL-C decreased 13%. Likewise, HDL-C decreased 8%. Plasma TG increased following increased carbohydrate intake, though the increase was not significant.

The AHA Step 1 diet limits cholesterol intake to 300 mg a day, contains 30% fat energy, 50-60% carbohydrate energy, and 10-20% protein energy. Although this level of fat intake remains higher than the consumption of fat in societies where the prevalence of ischemic heart disease is low (124), implementation of the Step 1 diet has resulted in successful reduction of atherogenic serum lipid levels in men and women (10, 129).

Hypercholesterolemic women who followed an American Heart Association Step 1 diet (30% fat/50-60% CHO) for eight weeks (129) averaged a 9.2% reduction in serum total cholesterol and serum LDL-C levels. Additionally, serum, HDL-C concentrations fell 3.6% and serum TG values dropped 11.3%. Interestingly, the Step 1 diet used in this study was equally effective in lowering serum total cholesterol, LDL-C, and the ratio of LDL-C to HDL-C in women as well as men. Those with the highest initial serum cholesterol levels appeared to achieve the greatest dietary response to dietary modifications (129).

A similar study has reported the efficacy of the Step 1 diet in hypercholesterolemic postmenopausal women (10). Study length was four months, twice the length of the study conducted by Geil et al (129). Despite this, the reported reductions of lipoproteins were less dramatic than those reported by Geil and colleagues (129). Total and LDL-C concentrations were significantly decreased. Average total cholesterol concentrations were reduced by 5% while LDL-C levels were decreased by

6%. While changes in plasma HDL-C and TG did not reach significance, each tended to decrease.

These findings may indicate that postmenopausal women are less responsive to moderate restrictions in fat intake. However, decreased subject adherence due to limited subject contact in this study may also explain the diminished responsiveness measured in this group. Geil and co-workers (129) provided group classes as well as individual visits biweekly to all subjects following initial instructions on the AHA Step 1 diet. Daily nutrient intakes were calculated based on three-day diet records completed at four timepoints throughout the study. Completed food frequency questionnaires were also utilized to confirm satisfactory compliance in this group. In contrast, researchers participating in Denke's study had subject contact on a monthly basis only. Furthermore, additional nutritional counseling was provided only to patients identified as having difficulty in achieving dietary goals. Lastly, nutrient intake was quantified using 7-day food records obtained during the last 2 weeks of the diet periods.

Effects of HCLF intake on HDL-C

While the major risk factors for CHD in women are similar to those for men, serum HDL-C concentration is a stronger CHD risk predictor than is serum LDL-C level. A low HDL-C (< 0.90 mmol/L or 35 mg/dL) concentration is the strongest lipoprotein/lipid predictor of coronary disease and myocardial infarction in women (3, 13, 130). Relationships between total, HDL-C, LDL-C, and TG were examined by Bass and colleagues (13) using data spanning a fifteen year period in women aged 50-69 years

of age who participated in the Lipid Research Clinics' (LRC) Follow-Up Study. Women with low HDL levels (<50 mg/dl) were more than three times as likely to die of CVD than women with high HDL levels ($\geq$ 50 mg/dl) at total cholesterol levels of 200 mg/dl or above. Their findings suggest elevated HDL levels may remove the increased risk of cardiovascular death generally associated with high total- or LDL-cholesterol levels.

Subsequent to menopause, women typically experience a decrease in HDL-C. Moreover, LDL-C increases and ensuing LDL-C concentrations actually become higher in post-menopausal women than in men of the same age. Thus, women's lipoprotein profile resembles that of men for approximately the last 30 years of life (130).

While diet modification to achieve a HCLF intake effectively reduces total and LDL-C levels, some scientists have expressed concern over reports of unfavorable decreases in HDL-C associated with these dietary modifications (8, 54, 122, 123, 126). Both carbohydrate intake and high polyunsaturated to saturated fatty acid ratios have been associated with decreases in plasma HDL-C concentrations in normal and hypercholesterolemic subjects (122). Unfortunately, there is little evidence that serum HDL-C level can be raised by dietary changes (3). However, those who are physical activity and do not smoke tend to have higher plasma HDL-C levels than individuals who are obese or smoke or are sedentary (3).

Although reductions in HDL-C following the consumption of a HCLF diet are not universal (124, 127, 131), it appears common. The consumption of simple carbohydrates (122) as well as complex carbohydrates (125, 126) have been associated with reductions in HDL-C concentrations. Reductions in plasma HDL-C have not only

been reported in response to formula feedings (8), but also following continued

consumption of conventional carbohydrate-containing foods (54, 125, 126).

Additionally, the reported reduction in HDL-C which persisted throughout a one year

period during subject adherence to HCLF intake removes the likelihood that this effect is

transient (125).

In defense of HCLF consumption, many have pointed out that inherently low

HDL-C along with low incidences of CHD are characteristic of populations which

chronically consume high carbohydrate diets (130, 132). Additionally, the LDL-C/HDL-

C ratio may actually be reduced if the LDL-C level falls in response to decreased

saturated fat intake that typically accompanies HCLF intake. Lastly, there is no evidence

that diets rich in complex carbohydrates, which lower both HDL-C and LDL-C levels are

associated with increased risk for CHD (3).

Effects of HCLF intake on plasma TG

While low HDL-C values are considered strong predictors for risk of atherogenic

disease, the relation between TG concentration and CHD is less clear (3). Elevated

plasma TG values have been found to be a univariate risk factor for CHD in many

epidemiologic studies, but this increase in risk is not independent of other lipid and

lipoprotein measures (133). For this reason, researchers have shown concern over

increases in plasma TG which frequently accompany HCLF intake (53, 54, 56).

Coulston and colleagues (54) reported elevated TG concentrations in healthy

subjects who consumed a HCLF (60% CHO/21% fat) diet for ten days. While the study

length may have been too short to detect a transient effect on plasma TG, persistent TG elevations have been observed in studies of greater duration (126, 134, 135).

The rate that carbohydrate intake is increased has been shown to play a role in TG response. Ullmann et al. (136) demonstrated that by gradually increasing the carbohydrate intake by 5% every ten days, consumption of a 65% carbohydrate diet produces only minimal changes in TG. Energy balance also affects endogenous TG production. Plasma TG generally decline when people lose weight; conversely, TG levels typically rise when weight is gained. This effect is independent of diet composition (137).

The effects of dietary carbohydrates on fasting TG is dependent on many variables. The type of carbohydrate appears to be important factor in determining carbohydrate's effect on fasting TG levels. In fact, an increase in fasting TG values was not observed in several different studies which employed diets containing large amounts of natural carbohydrates (131). Sucrose and fructose, in some circumstances, increase fasting TG more than do starch or glucose (137). The plant fiber content of HCLF diets may also attenuate or prevent the TG rise that generally occurs in the absence of fiber (138).

To determine the hypolipidemic effects of HCLF diets containing fiber, men with NIDDM were fed two weight-maintaining 70% carbohydrate diets for 9 to 14 days. One diet was high in plant fiber (64g) and the other was low in plant fiber (23g). Fasting serum TG values were higher in every patient at the completion of the HCLF diet without additional fiber than values at the completion of the HCLF with high fiber. The

high fiber HCLF diets produced only slight alteration in fasting serum TG values in contrast to the low fiber HCLF diets which resulted in significantly higher values.

The beneficial effect of more moderate intakes of fiber has been exhibited by both Rivellese et al. (127) and Cominacini et al. (131). Hyperlipidemic subjects who consumed 30g of fiber concurrent with a HCLF diet (55% CHO/27% fat) experienced significant reductions in TG levels at the end of a six month dietary intervention trial (127). Likewise, Cominacini and colleagues (131) reported significant decreases in plasma TG levels after 45 and 90 days of dietary treatment (60%CHO/24% fat) which included an average fiber consumption of 27g of fiber a day. In this study, the authors took additional measures to minimize the TG response to increased carbohydrate intake. Starch accounted for ~85-90% of total carbohydrate in the HCLF diet, resulting in lower sucrose intake than consumed in many studies which produced carbohydrate-induced HTG (54). Lastly, the HCLF diet emphasized foods that have a low glycemic index. While Cominacini and colleagues were not able to test the effect each of these factors had on fasting plasma TG alone, their findings do establish that with careful dietary management, fasting HTG that has been associated with increased carbohydrate consumption may be avoided.

SPECIFIC AIMS

The objective of this study was to determine if changes in plasma TG concentrations that occur in response to HCLF intake are related to the availability of the substrates originating from dietary carbohydrates or the substrates originating from lipolysis of adipose tissue.

Our hypothesi are that

1. Insulin sensitivity is not compromised subsequent to long-term increased carbohydrate consumption.

2. The changes in plasma TG subsequent to increased carbohydrate consumption are related to the intermediates of carbohydrate metabolism.

3. Changes in plasma TG in response to HCLF intake are not related to the increased availability of the lipolysis products, FFA and FG.

The approach to test these hypothesi are:

1. to determine the effect of long-term HCLF diet consumption on glucose oxidation by measuring plasma lactate and fasting RQ.

2. to examine the effect of HCLF intake on lipolysis through the analysis of the lipolysis products, plasma FFA and FG.

3. to investigate the effect of a stepwise increase in carbohydrate consumption on fasting plasma insulin and glucose concentrations.

4. to assess the effects of a stepwise increase in dietary carbohydrate intake on plasma lipoproteins and TG in postmenopausal women through the measurement of plasma total-, LDL-, and HDL-cholesterol, and TG, as well as the assessment of LDL-to HDL-cholesterol and total-to HDL-C ratios.

RATIONALE

As the leading cause of serious morbidity and mortality in women, CHD has a greater affect on the quality of life and independence of women in their later years than any other disease (15). Coronary heart disease (CHD) is responsible for the deaths of more than 250,000 American women each year (130, 139). During the past 30 years, many primary prevention studies, conducted virtually exclusively with men, have shown that plasma total cholesterol and LDL-C lowering are associated with a significant reduction in CHD morbidity and mortality in men free from CVD (14, 130). However, recent findings reveal that women may not respond similarly to the primary preventive measures effectively used in men (13, 15). The effectiveness of extending these findings to the female population warrants further investigation.

While the 1988 report of the NCEP recognized that being male was an independent risk factor for CHD, it neglected to identify the increased risk of CHD that postmenopausal women face. In their second report however, the Expert Panel formally recognized that being over the age of 55 is a positive CHD risk factor for females (2).

Given the impact CHD has on the population of the United States, primary prevention of CHD, which emphasizes risk factor reduction to prevent or retard the development of CHD disease, has become a major component of the NCEP guidelines (1, 2). The Adult Treatment Panel (ATP) of the NCEP has identified LDL-C as the major atherogenic lipoprotein and established that high levels of LDL-C are the primary target for cholesterol-lowering therapy (1, 2). Dietary therapy remains the first line of

defense in both primary prevention and the treatment of high blood cholesterol within the second set of NCEP guidelines (2).

The NCEP currently recommends that the general public limit the intake of total fat to less than 30% of calories, saturated fatty acids to less than 10% of calories, and cholesterol to less than 300 mg/d (1). These recommendations parallel the Step 1 diet which is the first line of defense used to lower high blood cholesterol levels (2). Recommendations to decrease cholesterol and fat intake are based on the finding that both dietary cholesterol and saturated fatty acids increase plasma LDL-C levels by down-regulating the LDL receptor (3).

Using data from female participants in the LRC Follow-Up Study, Bass and colleagues recently characterized the relationships between CVD death and serum lipid and lipoprotein concentrations in women aged 50 to 69 years of age. Their findings provide evidence that HDL concentration is a stronger predictor of CVD mortality in women older than 50 years than total cholesterol concentration. In fact, HDL-C was the most powerful predictor of cardiovascular death in women according to the LRC Follow-Up Study (13, 14). After adjustment for other CVD risk factors, HDL-C less than 50 mg/dL were strongly associated with cardiovascular mortality. Women with low HDL-C concentrations had a threefold increased risk of CVD death when compared with women with high HDL levels at all concentrations of total cholesterol above 200 mg/dL. Findings reported in both the Framingham Study and the Donolo-Tel Aviv Study reaffirm that the effects of HDL concentration on CVD risk outweigh the effects of total cholesterol level on CVD risk (13). Bass and colleagues also found that TG

concentrations were strong predictors of CVD death in age-adjusted and multivariate analysis data within the female population studied in the LRC Follow-Up Study. Women with HDL concentrations less than 50 mg/dL and TG levels greater than 400 mg/dL are at markedly increased risk of CVD death. Conversely, LDL- and total-cholesterol concentrations were poor predictors of CVD mortality. Examining Framingham data, Castelli also demonstrated an association of increased TG concentrations with an increased rate of CVD only in women whose HDL concentrations were less than 40 mg/dL (140). These findings suggest that current primary prevention measures targeted at decreasing total- and LDL-cholesterol may not effectively reduce the risk of CVD in women.

In light of the NCEP emphasis on diet changes to reduce CHD, these measures need to be investigated in women as well as men. Since dietary fat and carbohydrate are reciprocally related, recommendations to lower total fat are synonymous with recommendations to increase dietary carbohydrate. Increased consumption of carbohydrate has been associated with reductions in HDL-C concentrations in both men and women (54, 122, 125, 126). The results from both the Framingham and LRC Studies indicate that even modest changes in HDL concentrations could have profound effects on risk status for coronary disease in women (13). Furthermore, elevations in fasting plasma TG concentrations are commonly reported following continued consumption of HCLF intake (53, 54, 56). Findings from the LRC Follow-Up Study reveal that elevated TG levels are an independent risk factor for CV death in women, particularly in those who have low HDL levels (141, 142). Moreover, plasma HDL-C

and TG are frequently inversely related which may infer TG have an important role in the regulation of HDL metabolism (143). The possibility that TG has an important role in establishing the type, size, and quantity of HDL particles underlines the importance of understanding lipoprotein metabolism in women.

The primary goal of the present study was to examine the effects of HCLF intake on substrate utilization in postmenopausal women. In an effort to investigate the relationship between modifications in lipoproteins and substrate utilization, substrate oxidation and lipolysis variables were collected at baseline and following each stepwise-reduction in fat intake. Because scientific evidence suggests that the intracellular enzymes which process carbohydrates produce lactic acid as a function of metabolism (76, 80), plasma lactate was measured at each of these timepoints. A number of short-term HCLF studies have demonstrated that lactate concentrations are elevated following HCLF intake (18-21). Scientific evidence also indicates that lactate, which is a 3-carbon intermediate of glucose metabolism, is an important precursor and stimulator of lipogenesis *in vivo* (22, 23). These studies suggest that the consumption of excess carbohydrate may promote the formation of fatty acids via de novo lipogenesis and ultimately contribute to the elevation of plasma TG observed subsequent to HCLF consumption.

During the planning stages of our study, the National Institute of Health (NIH) funded a study designed to determine whether a biomarker for fat intake exists within the HDL-C fraction of the plasma lipids. The research design of the NIH project included a stepwise reduction in fat consumption and concurrent increase in carbohydrate occurring

over a 16-week period. The 16-week study length of the NIH project provided us with a unique opportunity to measure both transient and long-term effects of HCLF intake on substrate utilization and lipoproteins. Therefore, we were able to maximize our resources through collaboration with the NIH study. Postmenopausal women were identified to serve as subjects in the NIH-funded project. Thus, collaboration with this study afforded us the opportunity to study a population where the need for further research is universally acknowledged (3, 13, 14, 139). Two additional features of this study design were beneficial to the implementation of our study. The gradual, stepwise, increase in the proportion of carbohydrate provided to study participants enabled us to discount the possibility that elevations in TG were the product of sudden increases in dietary carbohydrate consumption (136). Lastly, the production and distribution of all study meals within the same facility improved our ability to achieve optimal patient compliance and assure quality control.

MATERIALS AND METHODS

Experimental Design

Our sixteen-week investigation implemented a stepwise reduction in fat intake using three diets which gradually replaced energy from fat with carbohydrate energy. The Cycle I diet was formulated to contain 35% fat. Cycle I lasted 4 weeks and was followed by Cycle II, which included 25% of the calories from fat and continued for 6 weeks. The final phase, Cycle III, allowed 15% of the calories as fat and also continued 6 weeks.

Subjects were studied in two separate groups consisting of sixteen subjects each. Of the initial 32 subjects, five subjects requested to be released from the study. Three of these subjects cited personal problems for their withdrawal while the other two subjects experienced family deaths during the study. Two subjects were excluded due to noncompliance. A total of 25 subjects completed the study. Each subjects served as her own control by participating in each of the three test periods. Subjects were instructed not to deviate from their regular activity habits during the 16 week study.

Recruitment of Subjects

Study subjects included healthy, postmenopausal women. Potential subjects were invited to an orientation meeting through telephone calls, personal letters, fliers, and newspaper advertisements. The objective of the orientation meeting was to obtain a group of potential study candidates. Additionally, the study design was explained in

detail, questions were answered, and all participants completed a background questionnaire (**Appendix B**). Questionnaires were reviewed to screen for eligible subjects. Individuals with known diabetes, systemic illness (renal, hepatic, gastrointestinal), hyperlipidemia (chol>300 mg/dL), cancer currently requiring therapy, or an unstable medical condition possibly requiring a change in medications were excluded. Although women not receiving hormone replacement therapy (HRT) were desired, women receiving continuous HRT or using a hormone replacement patch were accepted.

Following evaluation of completed questionnaires, qualifying participants signed an informed consent to participate which was approved by the Human Subjects Review Committee (HSRC), University of California, Davis (144) (**Appendix C**). These subjects then received a physical examination and provided a blood test which was used to assess blood glucose, plasma cholesterol, as well as liver and kidney function.

Dietary Intervention

Prior to the baseline diet period, subjects were instructed to record their food intake for a 1 week run-in period. The 7-day food records were analyzed by Nutritionist IV Software (version3.0, 1993 N-Square Computing, Silverton, OR). The energy requirement of each subject was based on their measured respiratory energy expenditure (REE) by indirect calorimetry and an activity factor was added. This value was then

compared with the caloric need calculated for the participants' age, sex, and activity level and adjusted as necessary (145).

Menus were formulated to provide 1600 calories. Unit muffins reflecting the energy composition of each cycle were provided to subjects who required greater than 1600 calories per day. Since all unit muffins contained 150 calories, caloric adjustments to the standard 1600-calorie menu were made in 150 calorie increments. To achieve weight maintenance, subjects were weighed every evening before dinner. Subjects were moved to the next caloric intake level if net weight change exceeded one kilogram or more on three consecutive days.

Test meals, administered in 7-day cycles, were prepared and served at the UCDMC Catering Facility. During the entire 16-week study, participants consumed dinner at the UCDMC Catering Facility. Breakfast and lunch, as well as all weekend meals were provided as take outs.

During the Intermediate and Strict Diet cycles, menu items used during the Baseline Diet period did not change but were adjusted to provide 25% and 15% fat intake, respectively (**Appendix D**). An attempt was made to formulate the three menu cycles to provide consistent ratios of cholesterol to fat and polyunsaturated to saturated fat intake. The cholesterol (mg) to total fat (g) ratio ranged from 2.99 to 4.68 during Cycles I, II, and III. Meanwhile, the polyunsaturated to saturated fat ratio ranged from 0.95 to 1.1. Attempts to keep the fiber content constant were also successful as illustrated by the small variation in fiber content of the three menu cycles. Cycles I, II, and III contained 9.1g, 11.4g, 10.5g fiber respectively. Subjects were instructed to limit

alcohol consumption to one alcoholic serving per week. One alcoholic serving was defined as 1 ounce of hard liquor, one can of beer, or a four-ounce glass of wine.

Compliance was monitored by inspecting trays for incomplete food consumption following each dinner meal. In an effort to optimize compliance, subjects were provided a list of "free foods" from which they could supplement the meals and snacks provided by the study **(Appendix E)**. Free items (e.g. diet soda, cucumber, sugar-free jelly), alcoholic beverages, and deviations from the prescribed dietary intake were recorded on the food records. The only foods subjects were allowed to consume in addition to that provided by the study included the items on the free list. Additionally, subjects were instructed to consume all food provided by the study. In the event that subjects consumed foods not provided by the study, they were instructed to record those items in their daily food records. Subjects were considered compliant as long as the amount of missed or added non-study food did not exceed 1% of total energy consumed.

Data Collection

All blood samples were drawn after subjects had fasted overnight for 12-14 hours. Blood samples were drawn at four timepoints: before the baseline diet period and following each test period. All samples were analyzed in duplicate and averaged.

Anthropometric data: Fasted subjects, dressed in hospital gowns and wearing no shoes, were weighed at the baseline, and at weeks 4, 10 and 16. The same scale was

utilized at each timepoint. Weights taken before dinner meals were taken with subjects in light clothing without shoes.

Percent body fat was determined using biometric impedance (Bioanologics, Beveton, OR) and triceps skinfold measurement.

Resting Energy Expenditure (REE) and RQ: REE and RQ were determined by indirect calorimetry using a constant velocity flow-through hood system (Thermox-Ametek, Pittsburg, PA). Testing occurred while subjects were in the fasted state, before breakfast, subsequent to at least 15 minutes of rest.

Plasma Lipoproteins: **Total cholesterol** (Sigma kit no. 352) and **plasma TG** (Sigma kit no. 336) were measured enzymatically using kits from Sigma (Sigma Diagnostics., St. Louis MO) The procedures used to determine total cholesterol are based on those developed by Allain et al.and colleagues (146). A modification of the method of Bucolo and David (147) was utilized to measure Plasma TG. **Plasma HDL** were determined by a serial precipitation method of Warnick (148), using dextran sulfate and magnesium chloride. **Plasma LDL-C and VLDL cholesterol** were calculated from the results of these tests using the following formulas (149):

VLDL cholesterol= TG/5

LDL-C = Total Cholesterol -(VLDL)-HDL-C

Other Biochemical Measurements: Due to their relationship to nutrient utilization, blood glucose and insulin were analyzed. **Blood glucose** concentrations were determined by the glucose oxidase method (kit no. 315, Sigma Diagnostics., St

Louis, MO) (149). **Plasma insulin** was determined by double antibody radioimmunoassay (^{125}I RIA kit, ICN Biomedicals, Ind., Costa Mesa, CA).

It has been suggested that lactate may provide a good marker of glucose storage (21). For this reason, **plasma lactate** concentrations were measured by an enzymatic test with lactate oxidase and peroxidase (kit no. 735, Sigma Diagnostics, St. Louis, MO), (Shimadzu uv-160 spectrophotometer, Kyoto, Japan) (150, 151). **Serum free fatty acids** were determined by the enzymatic colorimetric method of Wako Chemicals (kit no. 990-75401, Richmond, VA). Plasma free glycerol levels provide an indirect assessment of FFA/TG cycling generated by the adipose tissue. Therefore, **plasma free glycerol** was quantified fluorometrically according to the method of Boobis and Maughan (152) (Perkin-Elmer L5-50B fluorometer, San Jose, CA).

Quality Assurance: During each menu cycle, identical trays were prepared for the seven-day menu cycle. This food was homogenized and an aliquot was sent to the Hazelton Laboratories (Madison, WI) for analysis of the macronutrient composition.

Statistical Analysis: Results are expressed as means ± pooled SE for all variables at baseline and at the end of each of the three diet periods. Variables of interest were analyzed using repeated measures analysis of variance techniques using Statview 512+™ (Abacus Concepts, Inc., Berkeley, CA). When the ANOVA was significant (≤0.05), differences between dietary periods were compared with the use of the Fisher's paired t-test. Correlations were calculated with Pearson correlation coefficients.

RESULTS

<u>Subject characteristics</u> Baseline subject characteristics are presented in **Table 1**. During the study, thirteen of the 25 subjects were receiving either oral or transdermal hormone replacement therapy (HRT). With respect to alcohol consumption, subjects were allowed to consume one serving of alcohol per week; 10 subjects chose to incorporate alcohol into their diet according to these guidelines.

<u>Anthropometrics</u> Despite attempts to achieve weight maintenance, subjects lost an average of 2.3 kg during the 16-week intervention period (p=0.0001). The greatest amount of weight loss occurred during the first diet phase (35% fat diet) (p=0.0001). Body weight at all dietary periods was significantly different from baseline (**Table 2**). Likewise, BMI progressively decreased with each diet phase. Overall, subjects experienced an average decline in BMI of 3% during the entire intervention period (p=0.0001). Percent body fat was significantly reduced from baseline following Cycle I (35% fat diet) and remained at this level throughout the study. Reduction in percent body fat accounted for 74% of the weight loss.

<u>Nutrient composition of Cycles I, II, and III</u> In an effort to control for the divergent fat intake of our subjects prior to beginning the study, Cycle I menus were formulated to represent an average of their reported fat intake, which was ~35% of dietary energy. Analysis of the Cycle 1 diets revealed that the actual contribution of fat to energy composition was approximately 31% (**Figure 1**). In contrast, analysis of Cycles II and III showed that actual nutrient composition closely reflected menu formulations.

Table 1: Baseline Subject Characteristics

Variable[1]	Means ±SD	Range
Age at entrance (yrs)	58.5 ± 7.7	43-74
Weight (kg)	78.0 ± 16.8	53.3-116.8
Body mass index (kg/m^2)	30.49 ± 6.27	18.85-42.90
Body fat (%)[2]	33.8 ± 6.9	17.1-48.5
Fat Intake (% energy)[3]	32.8 ± 8.1	20-47
HRT[4]	13/25	
Alcohol Intake	10/25	

[1] n=25
[2] measured by bioelectrical impedance analysis
[3] 7-day food records analyzed by Nutritionist IV Software
[4] HRT, hormone replacement therapy, may include oral or transdermal hormone replacement of estrogen and/or progesterone.

Table 2: Changes in Anthropomentrics

Variable	Baseline[1]	Cycle I[2] (30% fat diet)	Cycle II (25% fat diet)	Cycle III (15% fat diet)	Treatment p-value
Weight (lbs.)[3]	171.62 ± 37.01^a	168.43 ± 36.46^b	167.51 ± 35.56^{bc}	166.85 ± 35.75^c	0.0001
Body mass index (kg/m^{2})	30.02 ± 6.35^a	29.48 ± 6.24^b	29.29 ± 6.07^c	29.17 ± 6.15^c	0.0001
Body fat (%)[4]	33.76 ± 6.89^a	32.66 ± 6.00^b	32.22 ± 6.27^b	32.74 ± 6.50^b	0.0008

[1] n=25
[2] Cycle I=30% fat, 52% carbohydrate, and 18% protein; Cycle II=23% fat, 60% carbohydrate, and 17% protein; Cycle III=14% fat, 67% carbohydrate, and 19% protein.
[3] values represented as mean ±SD
[4] measured by bioelectrical impedance analysis

Figure 1: Nutrient Composition of Cycles I, II, and III
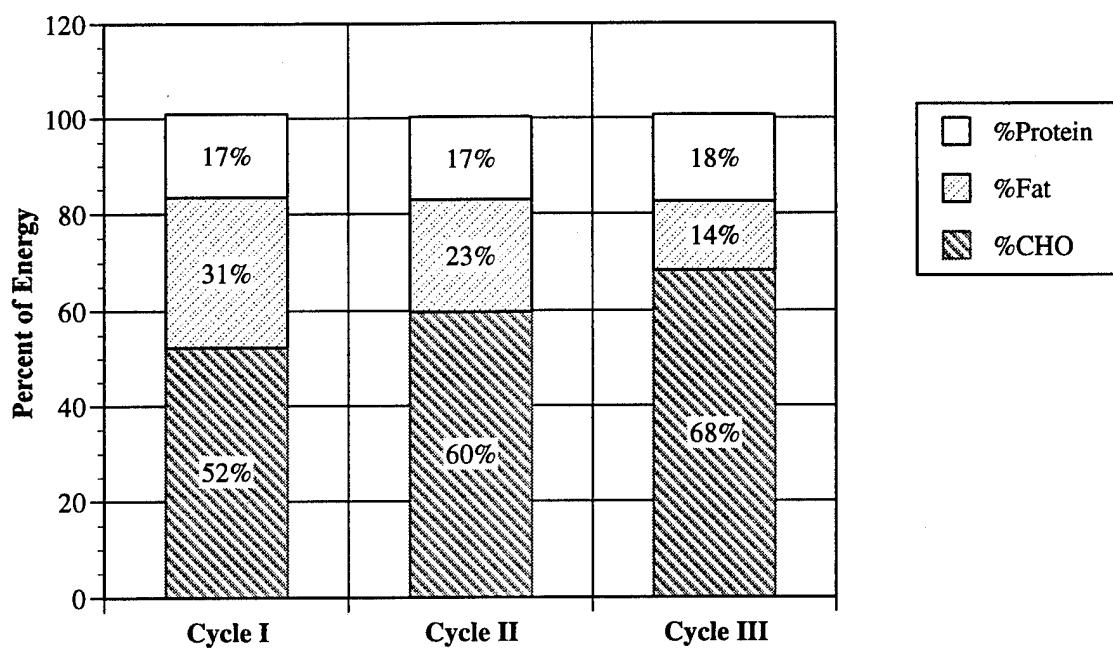
Cycle II (25% fat diet) derived 23% of the calories from fat and 60% from carbohydrate. In Cycle III (15% fat diet), actual fat content decreased to 14%; conversely, carbohydrate content increased to provide slightly more than 68% of the calories. Fiber content of the three menus remained fairly stable and averaged 10.3 g/1000 calories (**Table 3**). Although the cholesterol content inherently decreased with each reduction in fat, the cholesterol to fat ratio increased from 2.99 during Cycle I to 4.68 during Cycle III. The polyunsaturated to saturated fat ratio remained fairly constant across the three menu cycles, ranging from 0.95 to a.2.

Plasma lipoproteins Plasma lipids and lipoproteins and the ratios of total and LDL-C to HDL-C are shown in **Table 4**. Overall, the subjects experienced significant reductions in total cholesterol, LDL-C, and HDL-C following dietary intervention. In general, with each reduction in dietary fat, plasma lipids and lipoproteins were reduced in a stepwise fashion. Compared to baseline, total cholesterol was reduced 4% (p=0.0100), LDL-C 9% (p=0.0421), and HDL-C 14% (p=0.0001). In addition, consumption of the Cycle III diet (15% fat) was associated with a 36% elevation in both plasma TG (p=0.0006) and VLDL-C (p=0.0006). While there were differences between baseline and Cycle I, the differences between I and III were consistent with the changes observed between baseline and Cycle III. Interestingly, 21 of the 25 subjects had final TG values that were higher than their respective baseline values. Similarly, 21 subjects had final HDL-C values that were lower than their respective baseline values. Thus, increased carbohydrate intake produced a significant increase in the total cholesterol to HDL-C ratio while the LDL-C to HDL-C ratio remained unchanged.

Table 3: Fatty Acid, Cholesterol and Fiber Content of Cycles I, II, and III[1]

	Cycle I	Cycle II	Cycle III
Total fat (%)	31%	23%	14.2%
(g)	(34.5)	(25.7)	(15.8)
Saturated fat	8.2%	5.6%	3.3%
	(9.0)	(6.2)	(3.7)
PUFA	7.7%	6.5%	3.6%
	(8.5)	(7.2)	(4.0)
MUFA	11.1%	8.6%	5.5%
	(12.5)	(9.5)	(6.1)
P:S Ratio	0.95	1.2	1.1
Cholesterol (mg/1000 cal)	.103	89	74
Fiber (g/1000 cal)	9.1	11.4	10.5

[1] Diet analysis completed by Hazelton Laboratory, Madison, WI.

Table 4: Changes in fasting plasma lipids and lipoproteins

Variable	Baseline[2]	Cycle I[3] (30% fat diet)	Cycle II (25% fat diet)	Cycle III (15% fat diet)	Pooled SE	Treatment p-value
Total Cholesterol	5.95 (229)[a]	5.69 (219)[b]	5.81 (224)[ab]	5.69 (219)[b]	0.17 (6.43)	0.0072
LDL Cholesterol	3.22 (125)[a]	3.14 (122)[a]	3.12 (121)[ab]	2.93 (114)[b]	0.19 (7.36)	0.0425
HDL Cholesterol	1.95 (74)[a]	1.77 (68)[b]	1.73 (66)[bc]	1.66 (64)[c]	0.11 (4.28)	0.0001
VLDL Cholesterol	0.789 (30)[a]	0.747 (29)[a]	0.946 (36)[b]	1.08 (41)[c]	0.09 (3.54)	0.0001
Plasma Triglyceride	1.71 (152)[a]	1.62 (144)[a]	2.05 (182)[b]	2.34 (207)[b]	0.20 (17.70)	0.0001
Total Cholesterol/ HDLc	3.60[a]	3.60[a]	3.81[b]	3.86[b]	0.35	0.0091
LDLc/ HDLc ratio	2.12	2.14	2.21	2.12	0.31	0.7036

[1] units are mmol/L; units in parentheses are mg/dL.

[2] values expressed as means ± pooled standard error; n= 26. Groups not sharing superscripts indicate significant difference at $p<0.05$ between dietary treatments

[3] Cycle I=30% fat, 52% carbohydrate, and 18% protein; Cycle II=23% fat, 60% carbohydrate, and 17% protein; Cycle III=14% fat, 67% carbohydrate, and 19% protein.

Fasting glucose and insulin Fasting blood glucose and plasma insulin concentrations were significantly decreased from baseline compared to Cycle III (15% fat diet) (**Table 5**). Plasma insulin concentrations dropped significantly from 16 μIU/mL at baseline to 12 μIU/mL. In addition to the 24% reduction in plasma insulin, fasting glucose values decreased 7% during this time.

Measurements of lipolysis and substrate oxidation Consumption of the Cycle III diet (15% fat diet) tended to reduce plasma FG levels (**Table 5**). Although FG during Cycle III was not different from FG at baseline, FG following Cycle III was significantly lower than FG at both Cycles I and II ($p=.0030$, $p=0.0594$).

Interestingly, FFA concentrations were similar across dietary periods with the exception of Cycle II (25% fat diet) where FFA levels were lower than all other timepoints (**Table 5**).

Unlike the progressive reductions in the concentrations of metabolites (lipids, lipoproteins, FG) that reflect lipid metabolism, lactate concentrations rose steadily from Cycle I (30% fat diet) to Cycle III (15% fat diet) (**Table 5**). When compared to Cycle I (30% fat diet), lactate concentration increased 21% following Cycle II ($p=0.0088$), and then increased another 39% following Cycle III ($p=0.0065$).

Fasting RQ measurements did not change during the study period and RQ values remained between 0.83 and 0.86 (**Table 5**).

Fasting blood glucose, plasma insulin, FG, FFA, and lactate along with measures of obesity (percent body fat and BMI) were correlated with each other. Correlations of these variables at each of four timepoints are presented in **Table 6**.

Table 5: Changes in fasting metabolic measurements

Parameter[1,2]	Baseline	Cycle I[3] (30% fat diet)	Cycle II (25% fat diet)	Cycle III (15% fat diet)	Pooled SE	Treatment p-value
Glucose[4]	5.95 (107)a	5.75 (104)b	5.77 (104)ab	5.51 (100)c	0.15 (3)	0.0002
Insulin (μIU/mL)[5]	16a	13ab	13ab	12b	1	0.0164
Free Glycerol[5]	0.012ac	0.012ab	0.012ab	0.010c	0.001	0.1064
FFA (mEq/mL)[4,6]	0.874a	0.958a	0.712b	0.911a	0.058	0.0009
Lactate[5]	1.39 (12)a	1.12 (10)b	1.35 (12)ab	1.86 (17)c	0.10 (1)	0.0001
Respiratory Quotient[4]	0.850	0.832	0.833	0.860	0.019	0.4071

[1] units are mmol/L, unless otherewise noted; units in parentheses are mg/dL.
[2] values expressed as means ± pooled standard error. Groups not sharing superscripts indicate significant difference at $p<0.05$ between dietary treatments.
[3] Cycle I= 30% fat, 52% carbohydrate, and 18% protein; Cycle II= 23% fat, 60% carbohydrate, and 17% protein; Cycle III=14% fat, 67% carbohydrate, and 19% protein.
[4] n=24
[5] n=23
[6] FFA, free fatty acids

Table 6: Correlation coefficients of selected variables at four time periods

Parameters	timepoint	Body Fat (%)	Insulin	BMI	Free Glycerol	Lactate	FFA	Glucose
Body Fat (%)	Baseline	-	0.135	0.803****	0.420**	NS	0.052	NS
	Cycle I	-	0.220	0.868****	0.285	NS	0.048	NS
	Cycle II	-	0.347*	0.851****	0.406**	NS	0.341*	NS
	Cycle III	-	0.483**	0.858****	0.208	NS	0.104	NS
Insulin	Baseline		-	0.380*	NS	-0.046	NS	NS
	Cycle I		-	0.338	NS	0.385*	NS	NS
	Cycle II		-	0.353*	NS	-0.080	NS	NS
	Cycle III		-	0.538**	NS	0.156	NS	NS
BMI	Baseline				0.369*	NS	-0.068	NS
	Cycle I			⟨	0.152	NS	-0.061	NS
	Cycle II			⟨	0.330	NS	0.395*	NS
	Cycle III			⟨	0.019	NS	0.051	NS
Free Glycerol	Baseline				-	NS	0.500**	0.160
	Cycle I				-	NS	0.524**	-0.387**
	Cycle II				-	NS	0.204	0.137
	Cycle III				-	NS	0.592****	0.027
Lactate	Baseline					-	-	NS
	Cycle I					-	-	NS
	Cycle II					-	-	NS
	Cycle III					-	-	NS

BMI, body mass index; FFA, free fatty acids; NS, non significant; * $p < 0.10$, ** $p < 0.05$, *** $p < 0.005$, **** $p < 0.0005$

Percent body fat and BMI were positively correlated at every timepoint (p=0.0001). Thus, the higher the BMI, the greater the percent body fat. Percent body fat and BMI were also positively correlated with free glycerol. This relationship between FG and degree of obesity was the most apparent at baseline and Cycle II when the correlation between FG and percent of body fat was significant. Although there was no apparent relationship between BMI and FFA during the first two timepoints, the relationship reached significance during Cycle II (p=0.0509). Similarly, the relationship between percent body fat and FFA was the strongest during Cycle II. These relationships between FFA, BMI and percent body fat both lose their significance during the final diet phase. Neither percent body fat nor BMI was significantly correlated with lactate or glucose at any timepoint.

Plasma insulin correlated positively with percent body fat and BMI. The correlation between insulin and percent body fat was positive at each timepoint and reached statistical significance during Cycles II (p=0.0896) and III (p=0.0168). Similarly, plasma insulin was positively correlated with BMI and correlations reached statistical significance at baseline (p=0.0692) and Cycles II (p=0.084) and III (0.0067). Although a significant positive correlation existed between plasma insulin and lactate during Cycle I, there was no correlation between these variables at any other timepoint. We observed no significant correlations when plasma insulin was correlated with FG, FFA, or glucose at any timepoint.

The dependent relationship between plasma FG and FFA was reflected by the strongly positive correlation which was significant at all timepoints except Cycle II. This

relationship became highly significant during the final diet phase. The relationship between glucose and FG appeared to be somewhat random as it did not reach the usual level of statistical significance at any timepoint. Furthermore, no significant correlations existed between RQ and the variables measured (data not shown).

The degree of correlation between variables that may modulate plasma TG concentration were correlated with TG levels at each timepoint to evaluate the impact these factors have on TG concentration. While no consistent correlations were observed between selected variables and HDL or LDL, these data are presented in **Table 7**. Correlations of TG levels with plasma FFA, lactate, insulin, and BMI are shown in **Table 8**.

The correlation between fasting plasma TG and lactate was positive at each timepoint and reached statistical significance at baseline and Cycle III (p=0.0015, p=0.00574). The correlation between plasma TG and insulin was positive at three of four timepoints, but only in Cycle II did the association reach the usual level of statistical significance. While the relationship between plasma TG and FFA appeared somewhat random during the first three timepoints, plasma TG and FFA were significantly correlated following the final diet phase. Plasma TG and BMI remained positively correlated with each other through the intervention period. However, these correlations never reached statistical significance. We observed no significant correlations when plasma TG was correlated with FG, or glucose at any timepoint.

Figure 2 illustrates the interaction present between plasma cholesterol and BMI (p=0.0302). The cholesterol-lowering ability of low-fat/high-carbohydrate intake

Table 7: Correlation coefficients of HDL and LDL with selected variables at four timepoints

Parameters	timepoint	Lactate	Insulin	BMI	FFA
LDL	Baseline	NS	NS	NS	0.424*
	Cycle I	NS	NS	NS	0.151
	Cycle II	NS	NS	NS	0.113
	Cycle III	NS	NS	NS	-0.130
HDL	Baseline	NS	-0.243	NS	NS
	Cycle I	NS	-0.189	NS	NS
	Cycle II	NS	-0.436*	NS	NS
	Cycle III	NS	-0.104	NS	NS

BMI, body mass index; FFA, free fatty acids; LDL, low density lipoproteins; HDL, high density lipoproteins, NS, non significant.
*p<0.05

Table 8: Correlation coefficients of TG with selected variables at four timepoints

Parameters	timepoint	Lactate	Insulin	FFA	BMI	Free Glycerol	Glucose
TG	Baseline	0.622***	0.123	0.048	0.265	0.074	0.007
	Cycle I	0.317	0.440**	-0.134	0.243	0.088	0.255
	Cycle II	0.216	-0.061	0.206	0.246	0.013	-0.033
	Cycle III	0.385*	0.370*	0.443**	0.278	0.017	-0.154

BMI, body mass index; FFA, free fatty acids; LDL, TG, triglycerides.
*$p<0.10$, **$p<0.05$, ***$p<0.005$

Figure 2: Changes in Cholesterol by BMI

	Baseline	Cycle I	Cycle II	Cycle III
BMI <25	228	222	215	203
BMI=25-27	220	210	213	214
BMI>28	232	221	231	227
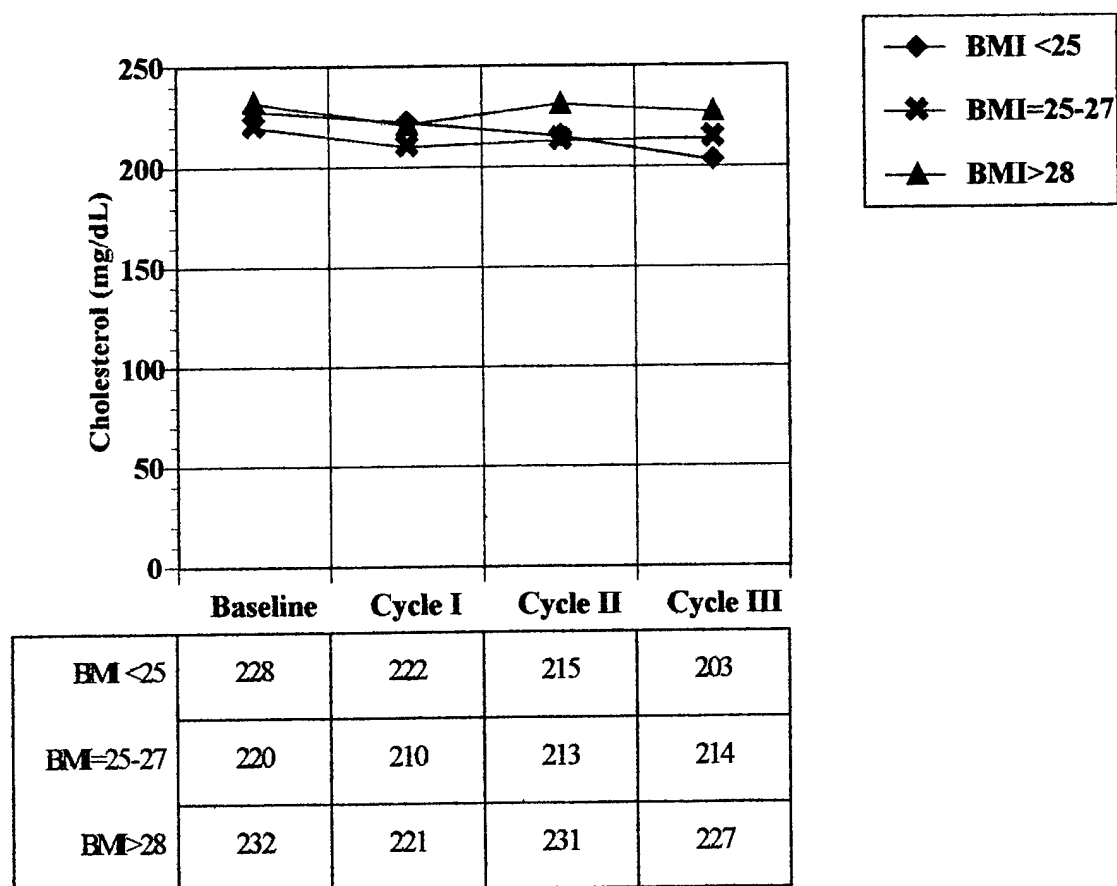
appeared to be the most effective in those subjects whose initial BMI was less than 28. The average net reduction of plasma cholesterol equaled 11% in subjects whose BMI was less than 25. By contrast, those whose BMI was greater than 25 at the onset of the study experienced only a 2 to 3% reduction in cholesterol concentration. There were no other interactions observed between BMI and lipids, lipoproteins, or other metabolic variables measured.

The change in plasma insuin which occurred between baseline and Cycle III was positively correlated with the simultaneous change in BMI (p=0.0619) (**Figure 3**). Similarly, a positive correlation exists between the change in plasma insulin and change in percent body fat which occurs between Cycles I and III. However, this relationship becomes significant only when two outliers (subjects #6 and #12) are excluded from the data set (p=0.0105) (**Figure 4**).

A significant positive association was present between the change in plasma lactate concentration and the change in percent body fat occurring between baseline and Cycle III (p=0.0209) (**Figure 5**). However, the relationship between these two variables was highly significant between Cycle I and Cycle III (p=0.0023) (**Figure 6**).

**Figure 3: Correlation of Change in Insulin to Change in BMI
Between Baseline and Cycle III**
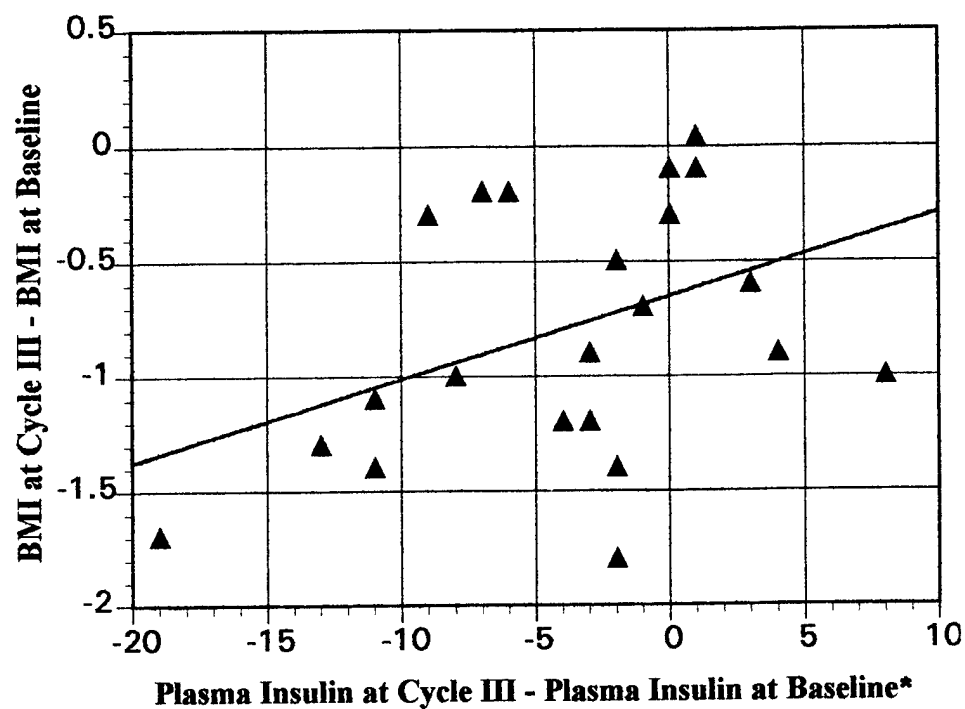
p = 0.0619

r = 0.395

n = 23

y = 0.36x - 0.654

* units are expressed as μIU/mL

Figure 4: Correlation of Change in Insulin to Change in % Body Fat Between Cycles I and III
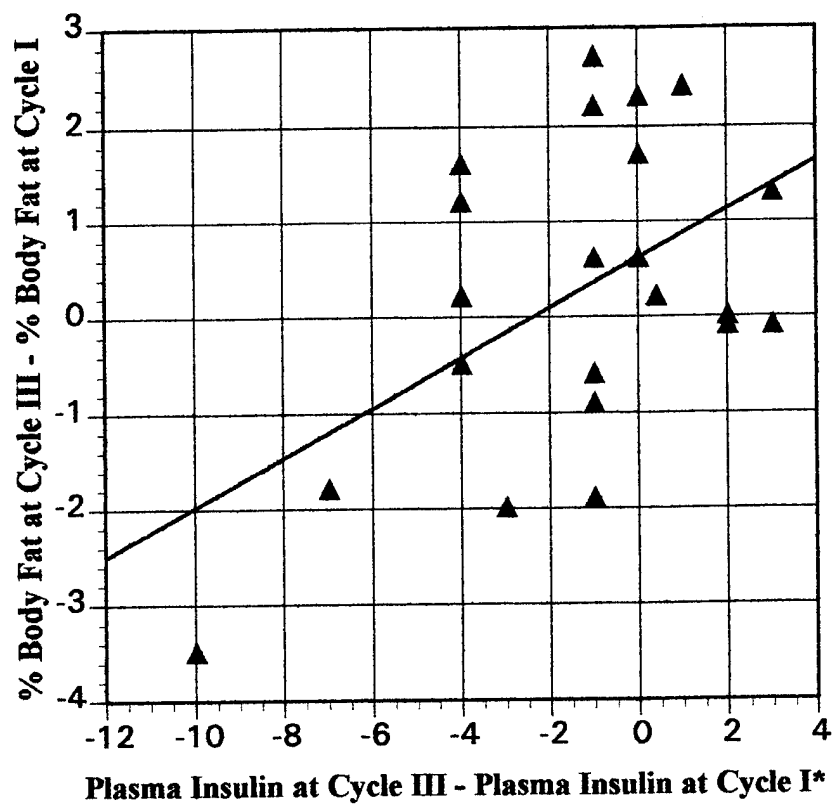
$r = 0.534$

$p = 0.0105$

$n = 22$

$y = 0.261x + 0.51$

* units are expressed as $\mu IU/mL$

Figure 5: Correlation of Change in % Body Fat to Change in Plasma Lactate between Baseline and Cycle III
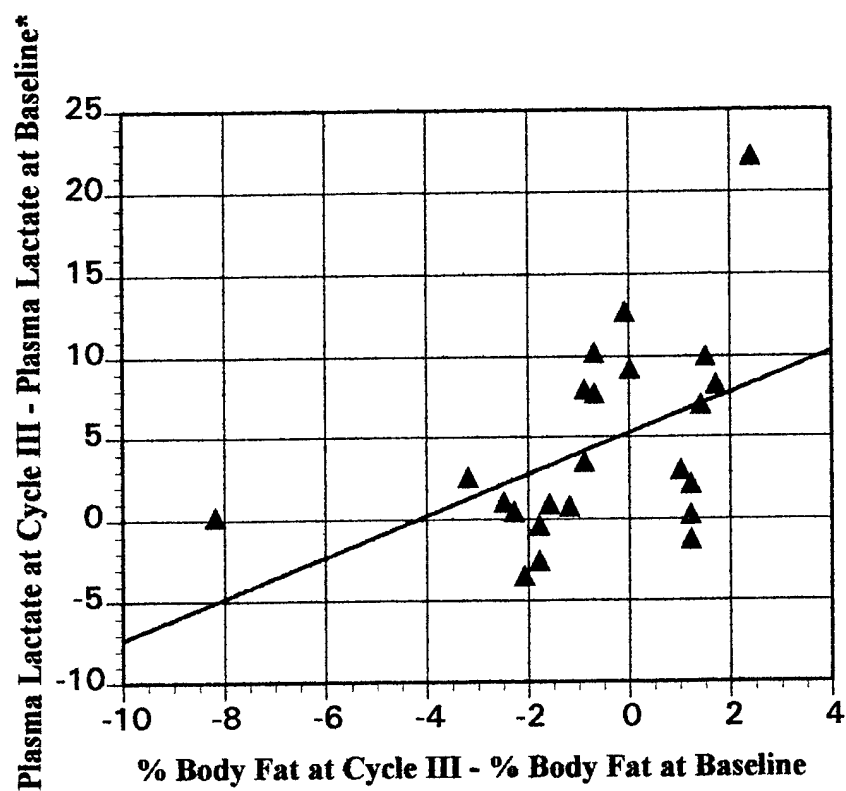
r = 0.478

p = 0.0209

n = 23

y = 1.259x + 5.286

* units are expressed in mg/dL

Figure 6: Correlation of Change in % Body Fat to Change in Plasma Lactate Between Cycles I and III
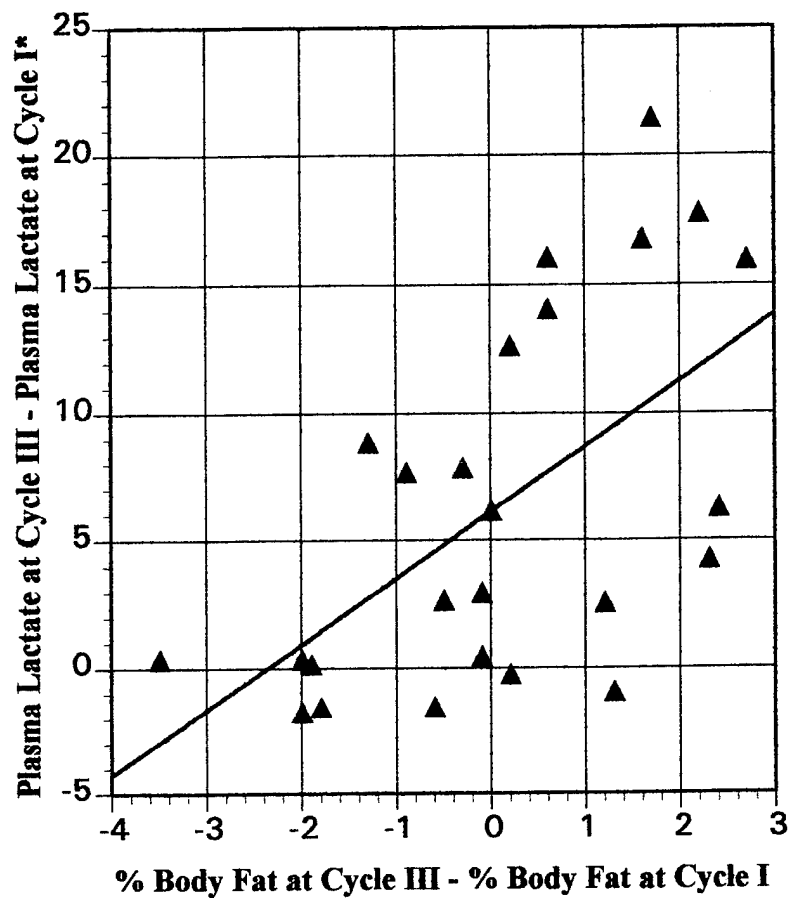
r = 0.582

p = 0.0023

n = 25

y = 2.584x + 6.112

* units are expressed as mg/dL

DISCUSSION

Effect of HCLF intake on anthropometric variables

Although diets for each subject were formulated to promote weight maintenance, weight loss did occur during the study. The greatest amount of weight loss resulted during Cycle I among the first group of women to complete the study. In an effort to minimize weight loss in group 2, we increased the activity factor used in conjunction with REE values to calculate the calorie needs of each subject in group 2. This proved helpful as average weight loss in group 2 was only 1.6 kg whereas subjects in group 1 lost an average of 2.9 kg.

Analysis of bioelectrical impedance data revealed that weight loss was accounted for primarily by reduction in fat mass (70%). While it has been suggested that weight loss is an inherent feature of low-fat diets (153, 154), studies in humans have not yet answered whether decreasing the proportion of energy as fat intake will affect weight loss or adiposity when total energy intake remains unchanged. This question was recently addressed in rats which were fed diets providing 75% of their previous ad libitum intake (155). Obese rats consuming 12% of their daily energy intake as fat lost significantly more weight and adipose tissue than rats consuming 45% of their daily energy needs as fat. Although this study indicates that dietary fat intake plays a key role in regulating both body weight and body composition, further human studies are needed to confirm that this occurs in humans. Because subjects typically restrict total energy intake when instructed to reduce fat intake, the effect of dietary fat intake on body

weight is difficult to separate from the effect of total energy intake (156). The decrease in energy consumption associated with HCLF intake has been attributed a number of variables. Many consider the palatability of a HCLF diet less desirable than that of a diet which contains more fat. Low-fat diets typically require a larger volume of food to be consumed in order to maintain energy intake. Likewise, HCLF diets are typically accompanied by an increase in fiber intake and it is possible that changes in fiber intake limit food intake by increasing satiety (156). We attempted to control the impact fiber may have on outcome variables by keeping fiber content of the cycle menus consistent throughout the study. The average fiber content of our three menu cycles ranged form 9.1 to 11.4 g fiber /1000 calories.

Effect of HCLF intake on insulin sensitivity

Following the 16-week intervention period, mean glucose concentration decreased 7% accompanied by a concurrent drop in insulin levels. Fasting insulin concentrations have been shown to correlate closely with insulin-mediated glucose uptake (157, 158). Based on this, insulin sensitivity appears to have been enhanced following continued HCLF intake.

Our findings agree with other research indicating that HCLF diets enhance insulin sensitivity (40-43, 45, 52). These studies have reported that diets containing more than 70% of dietary energy from carbohydrate are the most successful at improving insulin sensitivity. Insulin concentrations were significantly reduced from baseline both during Cycle III (70% CHO) and Cycle II when carbohydrate contributed only 60% of the

energy intake. At baseline and throughout Cycles I and II, a few individuals experienced fasting insulin values that are considered above normal (>4-25 μU/mL) (30). In contrast, all fasting insulin values were within normal limits subsequent to Cycle III.

The ability of HCLF diets to improve insulin sensitivity has been attributed to their fiber content as an increase in fiber intake typically accompanies HCLF intake (45, 47-50). In our study, however, the fiber content of the three diet cycles remained fairly constant, averaging just 10.3 g fiber per 1000 calories. Hence, fiber is not the only factor in HCLF diets that contribute to changes in insulin insensitivity.

Because plasma FFA concentration reflects, in part, an inhibition of lipolysis and stimulation of fatty acid re-esterification in fat cells by insulin, FFA levels provide another means to assess insulin effectiveness. The average FFA concentration of our subjects at baseline (0.860 mEq/mL) was somewhat higher than the expected normal range from fasting patients (0.1-0.6 mEq/mL) (159). However, expected normal ranges for FFA concentration may vary by population and locality (159). Based on our findings that plasma FFA following Cycle III were not different from those at baseline, it appears that subjects did not experience changes in insulin's effects on FFA concentration in response to long-term high carbohydrate intake. While we were unable to discount the effect of the small amount of weight loss experienced by our subjects, the improvements in insulin sensitivity noted appear to be in response to sustained consumption of HCLF diet (44, 112).

These conclusions regarding insulin sensitivity are difficult to reconcile with the hypothesis that the basic defect in those with HTG is resistance to insulin-mediated

glucose uptake (160, 161). Researchers have proposed that changes in insulin response elicited by increased carbohydrate consumption lead to compensatory hyperinsulinemia, increased synthesis and secretion of hepatic TG, and ultimately HTG. It has been established that increasing the amount of ingested carbohydrate results in elevated plasma insulin concentrations (11, 12, 43). However, our results reveal that plasma insulin does not remain elevated throughout the fasting state. In fact, fasting plasma insulin values were lowest during Cycle III, when carbohydrate consumption was the highest. This raises the possibility that subjects cycle between periods of normal and compromised insulin sensitivity. Nonetheless, using circulating levels of insulin to describe insulin sensitivity has been criticized (34). Therefore, further investigations that assess insulin sensitivity directly by the euglycemic clamp technique during both postprandial and fasting periods may clarify the effect HCLF intake has on the effectiveness of insulin.

Relationship between fasting insulin and anthropometric variables

Correlations between percent body fat and BMI were significant at every timepoint, indicating that body compositions between subjects were similar. Thus, within our study sample, percent body fat increased in parallel with BMI values and each subjects' degree of obesity was accurately reflected by their BMI. Out of 25 subjects, our study included 14 women whose BMI at baseline was greater than 28, generally indicative of obesity. These attributes reflect the fact that this subject population was quite sedentary and consumed a typical American diet. Thus, characteristics of this

sample should be kept in mind when interpreting results from this study and when comparing this study to other investigations.

It is well established that a modest weight loss of approximately 10% of initial body weight promotes increased peripheral insulin sensitivity (162, 163, 164). Although the average amount of weight loss experienced by our subjects was only 2.8% of initial body weight, the weight loss may have prompted a reduction in lipid oxidation leading to the enhanced oxidation of glucose, ultimately improving insulin sensitivity. This point becomes somewhat clearer when group 1 and 2 are compared separately. While group 1 experienced an average weight loss of 2.9 kg, group 2 lost 1.6 kg during the study. Moreover, plasma insulin concentrations in group 1 were significantly reduced from baseline following both Cycle I and Cycle III. In contrast, plasma insulin in group 2 was not significantly different from baseline at any timepoint. Furthermore, a significant positive correlation exists between the change in insulin and change in BMI which occurred between Cycle 1 and Cycle III. Since nearly all of the subjects lost weight during this period, this association may indicate that those subjects who lost the most weight experienced the greatest modification in fasting plasma insulin levels. This conclusion is supported by the positive correlation between change in insulin and change in percent body fat which occurred between baseline and Cycle III which is significant when two outliers are excluded.

The positive correlations between insulin and indicators of obesity (BMI and percent body fat) became significant only at the end of Cycle III, when body weight appeared to approach a steady state. The positive correlations between insulin and both

indicators of obesity (BMI and percent body fat) suggest that fasting insulin concentrations corresponded with the subjects' degree of obesity. The relationship between fat mass and insulin sensitivity has been elucidated by Groop and colleagues (44, 112). Their work demonstrates that in comparison to lean controls, subjects with uncomplicated obesity require higher insulin dosages for the stimulation of glycogen formation, inhibition of glucose production, and the suppression of FFA turnover. Furthermore, the suppression of FFA turnover by insulin in obese subjects is normal when expressed per kilogram fat mass. Consequently, increased insulin requirements may not indicate insulin insensitivity but simply an increased requirement due to excess substrate including an enhanced fat mass (112).

Effect of HCLF intake on measurements of lipolysis

Measurements of plasma FFA and FG have been used in a handful of studies to indicate the degree of postprandial lipolysis and reesterification occurring (25, 26, 58, 74, 75). Because the rate of fat oxidation generally varies in parallel with changes in the level of circulating FFA, FFA concentrations provide an indirect measure of lipid oxidation (36, 72). Free glycerol is liberated from TG along with FFA during lipolysis, thus, plasma FG concentrations also reflect the lipolytic activity in adipose tissue (165).

The effect of HCLF consumption greater than one week duration on plasma FFA and FG has not been reported to our knowledge. Thus, we were interested in measuring the changes in lipolysis that might occur when exogenous sources of FFA were decreased for an extended period of time. Both FFA and FG have been shown to

decrease following short-term HCLF consumption (25, 26, 74, 75). Following HCLF intake, lipolysis is reduced and de novo synthesis of TG from FFA is enhanced in response to insulin action. Additionally, the amount of FFA released from plasma TG is ultimately reduced since less fat is consumed (74, 75).

As expected, plasma FFA and FG concentrations were significantly correlated with each other throughout most of the study. It is not clear why these measurements did not correlate during Cycle II. Final plasma FFA and FG concentrations were not significantly different from baseline values, which may indicate that lipolysis was not affected by dietary intervention. Given the degree of influence obesity has on plasma levels of FFA and FG, we were surprised that plasma FG and FFA values were not significantly correlated with BMI and percent body fat at all timepoints. Our samples were drawn during fasting and thus, may not accurately represent typical FFA or FG levels experienced throughout the day. FG and FFA turnover would provide a more accurate reflection of actual lipolysis rate. Because data regarding the effects of HCLF intake on FFA and FG concentration are lacking, comparisons are difficult to make.

Effect of HCLF intake on measurements of substrate oxidation

It is well established that lactate is produced by erythrocytes and fast twitch (white, glycolytic, type IIB) muscle fibers which function under hypoxic conditions (76, 77). Because erythrocytes and fast twitch muscle are lacking mitochondria which contain the enzymatic machinery for the aerobic oxidation of pyruvate, glycolysis in these tissues always terminates in lactate, even in aerobic conditions. However, it has

recently been suggested that the intracellular enzymes that process carbohydrates produce lactic acid as a function of metabolism regardless of oxygen availability. Studies on dog gracilis muscle *in situ* provide additional evidence that lactate production occurs in contracting slow-twitch (red, Type I) muscle in the presence of an adequate oxygen supply for mitochondrial ATP production (80). The mechanism by which lactate is produced under fully aerobic conditions is frequently referred to as the lactate shuttle. Once produced, lactate may undergo one of three possible metabolic fates: oxidation via the citric acid cycle, gluconeogenesis, or conversion to acetyl-CoA to support lipogenesis (166). Thus, lactate formation provides the organism with a great deal of metabolic flexibility. Given that lactate possesses the ability to be oxidized or serve as a substrate for either gluconeogenesis or lipogenesis, the existence of a lactate shuttle may have far-reaching metabolic implications.

The existence of a lactate shuttle suggests that our present understanding of lactate metabolism may be incomplete. Historically, the presence of lactate has served only as an indicator of an inadequate oxygen supply (76, 80). However, a review of the available literature on HCLF feeding trials reveals that elevations in plasma lactate concentration have frequently been associated with increased carbohydrate intake (18-21, 82, 83). Consequently, this diverse metabolic substrate may also serve as a marker of glucose storage. In studies of both rat and mouse hepatocytes, lactate appears to be a strong promoter of lipogenesis through both its stimulatory action and its ability to serve as a lipogenic precursor (22, 23, 167). The common association of HTG and hyperlactatemia with HCLF intake suggests that enhanced de novo lipogenesis from

lactate provides a significant contribution to the HTG that frequently ensues following HCLF intake. Nonetheless, support for this proposal is limited in that only the short-term effects of HCLF consumption on fasting plasma lactate levels have been measured. To our knowledge, we are the first to report the long-term effects of HCLF intake on plasma lactate concentrations.

The significant rise in fasting plasma lactate following long-term HCLF consumption is similar to elevations in plasma lactate reported previously in short-term HCLF trials (18-21). While previous studies established that plasma lactate is elevated postprandially following HCLF consumption, we have shown that fasting plasma lactate levels are similarly elevated by long-term HCLF intake. These findings support the existence of a lactate shuttle and suggest that lactate may even be a marker of glucose storage.

Interestingly, the strong association between decline in lactate and decline in percent body fat suggests that in subjects who experienced reduction in body fat, glucose was utilized as oxidizable substrate and not recycled into lactate. Conversely, in subjects who were not in a calorie deficit state and did not lose body fat, glucose was recycled into lactate and thus concentrations were increased.

RQ measurements remained unchanged throughout the dietary intervention periods. The RQ measurements in this study were taken while subjects were in a fasting state. Thus, it is reasonable that fasting RQ values did not change despite increased carbohydrate consumption. However, it is also possible that the RQ values we measured were not an accurate reflection of the subjects' substrate utilization. During RQ testing

at baseline, it was evident that some subjects had difficulty becoming accustomed to the RQ testing procedure, leading to hyperventilation and inflated baseline RQ values in a number of subjects. Although subsequent RQ values may have actually increased in response to successive carbohydrate augmentation, these changes would have been difficult to detect.

Plasma ketone measurements provide a method to quantify fatty acid oxidation (168). The ketone body, β-hydroxybutryrate (BHB), is formed almost exclusively in the liver secondary to fatty acid oxidation (18). Thus measurement of BHB provides a quantifiable indicator of hepatic fatty acid oxidation. Although we attempted to quantify plasma ketones, these values will not be reported due to technical difficulties. The nature of our difficulty in completing this assay was attributed to the low concentration of BHB in the samples collected, making detection of BHB especially difficult. Based on the assays that were completed, we were convinced that oxidation of fatty acids was not excessive and thus, plasma ketone concentration was within normal range.

Effect of HCLF intake on total cholesterol and lipoprotein metabolism

Based on metabolic-ward studies, the Expert Panel predicts an average reduction in total cholesterol of 45-55 mg/dL (1.2-1.4 mmol/L) with adherence to the NCEP Step 2 diet which limits daily consumption of saturated fatty acids to less than 7% of calories and cholesterol intake to less than 200 mg. During our study, saturated fatty acid intake was reduced to 8.2, 5.6 and 3.3 % of daily energy intake during Cycles I, II, and III, respectively. Additionally, Cycle I provided only 103 mg of dietary cholesterol per day

and this value was reduced 25% further during Cycles II and III. Although our dietary regimen was more restrictive than the prescribed Step 2 guidelines, total cholesterol levels in the present study were reduced an average of only 10 mg /dL or 4% of baseline values. Our results are consistent with those reported in previous diet trials that reduced fat intake in women to 15-21% of daily energy consumption for a period of five to twelve months (9, 10, 12).

A number of factors are likely to have contributed to the discrepancies observed in cholesterol responsiveness following dietary modifications to reduce fat intake. First, the NCEP predictions were based on metabolic ward-studies where dietary intake can be more readily controlled than in most outpatient settings. Our subjects were instructed to record all the foods consumed not provided by the study on a daily food record which was collected weekly. Additionally, we had daily contact with our subjects (Monday through Friday) and were able to monitor subjects during the evening meals to assure all foods provided by the study were consumed. Based on our interaction with the subjects at the evening meals and subjects' daily food records, we feel that the compliance of each subject in our study was satisfactory. Secondly, the variance in cholesterol lowering may be related to BMI. It has previously been reported that obese women are less diet sensitive than lean women (9). In this study, a significant interaction was present between BMI and the effect of HCLF intake on plasma cholesterol concentrations. The magnitude of cholesterol reduction was diminished in those subjects with a BMI greater than 25 at baseline in comparison to women whose BMI was <25 at baseline. To illustrate, cholesterol concentrations fell 11% among those whose BMI was

<25 at baseline. In comparison, those whose BMI at baseline was >25 experienced only a 2-3% reduction in plasma cholesterol. This finding provides additional strength to the conclusion that obese women are less responsive to diet modification than lean women. Finally, a collection of studies indicates that women may be less responsive to dietary modifications aimed at improving cardiovascular risk than are men (9, 126, 169). This conclusion remains controversial as illustrated in a recent study comparing the effects of a Step 1 diet on serum lipid levels in men and women. Authors of this study concluded that adherence to the Step 1 diet was equally effective in reducing total cholesterol in women and men (129). Further study is required to elucidate those factors which produce variable responsiveness to dietary modification. Additionally, there is a paucity of scientific literature pertaining to differences in dietary responsiveness of premenopausal and postmenopausal women. Although it is well-established that women experience a decrease in HDL-C subsequent to menopause (130), it is not well understood whether postmenopausal changes affect cholesterol-responsiveness to dietary changes.

In addition to producing significant reductions in total- and LDL-cholesterol, the stepwise reduction of fat utilized in our study also produced a significant 14% reduction in mean HDL-C concentration. The introduction of the Cycle I diet, containing a moderate proportion of fat (31%), resulted in a rapid reduction in HDL-C level which did not return to initial levels during the remaining phases of the study.

Because the LDL- and HDL-cholesterol concentrations were decreased by approximately the same percentage, the ratio of LDL- to HDL-cholesterol did not

change significantly following dietary intervention. Conversely, the total- to HDL-cholesterol ratio was 7% higher than baseline following dietary intervention.

The above changes in total cholesterol and lipoproteins are consistent with those reported in previous diet trials that reduced fat intake in women to 15-30% of daily energy consumption (9, 10, 12,129). From these studies, it appears that women respond to HCLF diet modifications with reductions in total, LDL-, and HDL-cholesterol concentrations. Additionally, the mean cholesterol to HDL-C ratios in each of these studies generally increased adversely (12) or remained at baseline values in response to diet modification (9, 10). Moreover, the following discussion provides evidence that HDL-C and total cholesterol/HDL-C ratios may be more indicative of cardiovascular risk in women than LDL-C or total cholesterol levels. For these reasons, the benefits of HCLF diets are currently under scrutiny (8, 12, 53, 54, 170).

While it has been conclusively demonstrated that total, HDL, AND LDL-C levels are independent predictors of CVD in men, considerably less information is available regarding the effect different lipid profiles have on disease in women. Only three studies, the Framingham Study (171), the LRC Follow-Up Study (172) and the Donolo-Tel Aviv Prospective Coronary Artery Disease study (173) have evaluated lipoprotein levels as predictors of CVD and CVD death in women. Although the Framingham Study indicated that LDL concentration was positively associated with CVD in women, (171) this association was not supported by the LRC Study (13) or in a recent analysis of LRC Follow-Up Study data (13). Thus, the role of LDL as a predictor of CVD in women has been difficult to defend. In contrast, all three studies previously identified have

demonstrated a strong, independent, inverse association of HDL level with CVD risk in women. Additionally, analysis of the LRC Follow-Up Study data supports these observations and provides further evidence that HDL level is a stronger predictor of CVD mortality in women older than 50 years than total- and LDL-cholesterol levels (13). Furthermore, the increased risk generally associated with elevated concentrations of total or LDL-cholesterol are reportedly neutralized when HDL-C levels are high (13). The appropriateness of emphasizing LDL-C levels in CHD risk reduction therapy was also examined in a comparison of the ability of total cholesterol, LDL-C ratio of total cholesterol to HDL-C, and the ratio of LDL-C to HDL-C to predict the development of CHD in men and women (174). Findings from this study suggest that the cholesterol/HDL ratio provides a superior device to discriminate CHD risk in both men and women than either total- or LDL- cholesterol concentration.

At present, there is no evidence that diets rich in complex carbohydrates which lower both HDL-C and LDL-C levels are associated with increased risk for CHD (3). Additionally, populations which chronically consume high carbohydrate diets are characterized by inherently low HDL-C concentrations as well as low incidences of CHD (130, 132). However, the above findings emphasize the critical need for continued research to investigate the appropriateness of current dietary recommendations aimed at reducing CVD risk in women.

Effect of HCLF intake on plasma TG concentrations

Recent investigations have suggested that HCLF diets may accentuate HTG (53, 54, 56). This finding could have deleterious consequences in women since prospective studies indicate elevated TG concentrations are an independent risk factor for cardiovascular death in women (13). Additionally, HTG appears to be an even stronger predictor of CVD risk in women than in men (13, 15).

Ullman and colleagues have demonstrated that the dietary induction of HTG is largely prevented when carbohydrate intake is gradually increased over a period of time (136). Based on these conclusions, the present study which increased carbohydrate intake in a stepwise fashion should have minimized the development of HTG. Despite these assumptions, plasma TG and VLDL-C were significantly higher than baseline following Cycles II and III. We recognize that the incorporation of carbohydrate was somewhat accelerated in our study design relative to that utilized by Ullman and colleagues. However, we feel that the degree of TG response in this study reveals that there are other variables involved in the modulation of carbohydrate-induced HTG than the rate at which carbohydrate is introduced into the diet.

To illustrate this point, TG concentrations have reportedly dropped significantly in subjects who increased the proportion of carbohydrate consumed from 40% to 60% of their energy requirement (131). These subjects simultaneously increased their average fiber consumption to 27 g fiber per day as the amount of calories from carbohydrate replaced those from fat. Additionally, sucrose provided a much smaller contribution to caloric intake than in many studies which have produced carbohydrate-induced HTG

(54). Lastly, foods that have a low glycemic index were emphasized. Weight loss may also provide an explanation for the absence of HTG development in some HCLF studies (124). Subjects who adhered to a very HCLF (75% CHO/10% fat) diet for three months experienced no change in plasma TG concentrations (124). The average decrease in body weight during this period was 7.4 kg. Thus, it appears that weight maintenance during HCLF intake in subjects who are overweight may also contribute to carbohydrate-induced HTG. Although the effect each of these factors has on fasting plasma TG alone has not been clarified, the present study introduces a number of variables which may impact the degree of hypertriglyceridemic response observed following dietary modification which increases carbohydrate intake.

Relationship between plasma TG and measures of lipolysis

The correlation between plasma FFA and TG concentrations became significantly correlated only after Cycle III, the cycle which contained the greatest proportion of carbohydrate. This correlation between plasma FFA and TG suggests that subjects who experienced the highest FFA levels consequently had the highest TG levels. It is interesting to note that although plasma FFA were not different at Cycle III from baseline, there was no correlation between plasma FFA and TG at baseline. These results point out that measuring circulating FFA concentrations is not adequate to detect the magnitude of changes incurred by lipolysis, re-esterification, and de novo lipogenesis.

The presence of an enlarged fat mass, as seen in obesity, has been associated with increases in plasma FFA (72, 110, 111). Evidence is available to show that glucose

metabolism can be inhibited when FFA concentrations are elevated (36, 64). For this reason, a significant correlation between degree of obesity and carbohydrate induction of HTG was anticipated. However, our findings do not confirm this relationship as there were no significant correlations between fasting plasma TG concentration and BMI at any timepoint. In a recent cross-sectional study which included relatively sedentary postmenopausal women, abdominal obesity was directly associated with TG concentrations, whereas overall obesity was not related. (175). The authors reason that women with upper-body obesity experience higher insulin concentrations and greater turnover of plasma FFA, both of which stimulate hepatic VLDL TG synthesis. Among the 25 subjects enrolled in the present study, 15 women (60%) were characterized by upper-body obesity, identified by the presence of a waist to hip ratio greater than 0.8 (176). However, we found no association between plasma TG concentration and upper body obesity (determined uisng waist to hip ratios) at any timepoint.

Relationship between plasma TG and lactate concentrations

Our study shows that carbohydrate-induced HTG is accompanied by a concurrent increase in plasma lactate concentrations. Following a 16-week stepwise increase in carbohydrate intake, both plasma TG and lactate concentrations were markedly elevated from baseline. Although lactate concentrations rose when carbohydrate intake provided 60% of dietary energy, it was not until carbohydrate provided close to 70% of the caloric intake that individual subjects experienced lactate levels considered above normal (16 mg/dL) (30). Conversely, four subjects experienced

HTG (TG>200 mg/dL) prior to dietary intervention (2). Moreover, two additional subjects became hypertriglyceridemic following Cycle I which provided just 53% of caloric energy from carbohydrate. These findings indicate that increases in plasma TG closely parallel carbohydrate intake while abnormally high plasma lactate concentrations are most likely to occur when carbohydrate intake approaches 70% of caloric intake. These findings also suggest that the stimulus for HTG may be multifactorial.

Plasma lactate and TG were significantly correlated at two of four timepoints. Hence, plasma TG concentrations were higher in those with higher plasma lactate levels at these timepoints. These findings suggest an important role for lactate in lipogenesis *in vivo*. The increased peripheral utilization of glucose, which occurs in response to increased carbohydrate intake, results in the increased production of the three-carbon glycolytic intermediates, lactate and pyruvate. Several in vitro experiments (22, 23, 177) have demonstrated that lactate carbon is a significant precursor of FFA and therefore of TG. Moreover, lactate has been shown to stimulate the incorporation of FFA into the TG molecule and to inhibit the oxidation of lipid (177). Furthermore, there is evidence to demonstrate that lactate stimulates the hormonal induction of lipogenic enzymes (167). Therefore, it seems likely that lactate may be important both as a precursor and as an indirect stimulator of lipogenesis. If this is true, the production of lactate induced by continued HCLF intake may be an important factor in the development of HTG. Additional research is warranted to determine the long-term implications that increased production of lactate, a lipogenic substrate, may have on overall HTG and CVD risk in postmenopausal women.

SUMMARY

In an effort to minimize CVD risk, a number of national advisory groups have issued recommendations to increase carbohydrate consumption (2-6). However, clinical trials have shown that continued HCLF intake is often accompanied by elevations in fasting TG and reductions in HDL-C, which may have deleterious affects on CVD risk (8-12). Moreover, plasma TG and HDL-C have been identified as superior markers of CVD risk in women when compared to total- and LDL-cholesterol concentrations (13-15). For these reasons, the benefits of increased carbohydrate consumption are being questioned (8, 12, 53, 54, 170).

To enter this discussion appropriately, it is important that we understand the metabolic processes underlying the changes observed in lipid and lipoprotein profiles subsequent to continued HCLF intake. We hypothesized the adverse modifications in lipoprotein profiles associated with increased carbohydrate consumption may be related to changes in substrate oxidation. To elucidate these changes in substrate utilization, we measured RQ, fasting plasma insulin, glucose, lactate, FFA and FG at four different timepoints throughout a sixteen-week stepwise reduction in fat intake.

Prolonged carbohydrate consumption by our subjects produced concurrent decreases in total-, LDL-, and HDL-cholesterol concentrations which were accompanied by both an increase in plasma TG and cholesterol to HDL-C ratio. The amount of cholesterol reduction reported in this study was noticeably less than would be predicted with adherence to the NCEP Step 2 diet. However, our results were consistent with

those reported previously in diet trials that reduced fat intake in women to 15-21% of daily energy consumption for a period of five to twelve months (9, 10, 12). These findings emphasize the need for continued research in postmenopausal women to identify the most effective nutritional therapy in minimizing CVD risk in this population.

Following the stepwise reduction in fat intake, mean fasting glucose and insulin concentrations were decreased. Based on fasting plasma insulin, glucose, and FFA concentrations, insulin sensitivity appeared to improve in response to continued HCLF intake. Plasma FFA and FG concentrations indicate that lipolysis was not affected by dietary intervention throughout the study. RQ measurements were unchanged throughout the dietary intervention periods.

In contrast, fasting plasma lactate levels were elevated subsequent to continued HCLF intake, which is in agreement with the presence of a lactate shuttle. The parallel decline in lactate and percent body fat suggests that lactate provides an important source of metabolic energy. These findings suggest that lactate serves an important role as a diverse metabolic substrate.

Our study further demonstrates that carbohydrate-induced HTG is accompanied by a concurrent increase in plasma lactate concentrations. The strength of this relationship was reinforced by a significant correlation between plasma lactate and TG at two of four timepoints. Moreover, a collection of *in vitro* experiments provide strong support to the plausibility that lactate has an important role both as a precursor and as an indirect stimulator of lipogenesis (22, 23, 167, 177). The production of lactate induced by continued HCLF intake and its role in lipogenesis *in vivo* may be important factors in

the development of HTG. Additional research is warranted to determine the long-term

implications increased production of lactate, a lipogenic substrate, may have on CVD

risk in postmenopausal women.

LITERATURE CITED

1. The Expert Panel. Report of the National Cholesterol Education Program Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults. *Arch Intern Med* 1988; 148:36-69.

2. The Expert Panel. Summary of the second report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. *JAMA* 1993; 269:3015-23.

3. American Heart Association. Rationale of the diet-heart statement of the American Heart Association. *Circulation* 1993; 88(6):3008-29.

4. American Diabetes Association. Nutrition recommendations and principles for people with diabetes mellitus. *Diabetes Care* 1994; 17:519-22.

5. The Nutrition Committee, American Heart Association. Dietary guidelines for healthy American adults. *Circulation* 1988; 77:721A-724A.

6. U.S. Department of Health and Human Services. *The Surgeon General's Report on Nutrition and Health.* Washington, DC, U.S. Department of Health and Human Services, Public Health Service, U.S. Government Printing Office, 1988.

7. Little JA, Graves K, Suchindran CM. Customary diet, anthropometry, and dyslipoproteinemia in selected North American populations: The Lipid Research Clinics Program Prevalence Study. *Circulation* 1986; 73(suppl I):I-80-I-90.

8. Gonen B, Patsch W, Kuisk I, Schonfeld G. The effect of short-term feeding of a high carbohydrate diet on HDL subclasses in normal subjects. *Metabolism* 1981; 30(11):1125-9.

9. Cole TG, Bowen PE, Schmeisser D, Prewitt TE, Aye P, Langenberg P, Dolecek TA, Brace LD, Kamath S. Differential reduction of plasma cholesterol by the American Heart Association Phase 3 Diet in moderately hypercholesterolemic, premenopausal; women with different body mass indexes. *Am J Clin Nutr* 1992; 55:385-94.

10. Denke MA. Individual responsiveness to a cholesterol-lowering diet in postmenopausal women with moderate hypercholesterolemia. *Arch Intern Med* 1994;154:1977-82.

11. Reaven GM, Olefsky JM. Increased plasma glucose and insulin responses to high-carbohydrate feedings in normal subjects. *J Clin Endocrinol Metab* 1974; 38:151-3.

12. Ginsberg H, Olefsky JM, Kimmerling G, Crapo P, Reaven GM. Induction of hypertriglyceridemia by a low-fat diet. *J Clin Endocrinol Metab* 1976; 42:729-35.

13. Bass KM, Newschaffer CJ, Klag MJ, Bush TL, Bush TL. Plasma lipoprotein levels as predictors of cardiovascular death in women. *Arch Intern Med* 1993; 153:2209-16.

14. Bush TL, Fried LP, Barrett-Connor EB. Cholesterol, lipoproteins, and coronary heart disease in women. *Clin Chem* 1988; 34(8B):B60-B70.

15. Judelson DR. Coronary heart disease in women: risk factors and prevention. *JAMWA* 1994; 49(6):186-97.

16. Elwyn DH, Gump FE, Munro HN, Iles M, Kinney JM. Changes in nitrogen balance of depleted patients with increasing infusions of glucose. *Am J Clin Nutr* 1979; 32:1597-611.

17. Leitch CA, Jones PJH. Measurement of human lipogenesis using deuterium incorporation. *J Lipid Research* 1993; 34:157-63.

18. Surina DM, Langhans W, Pauli R, Wenk C. Meal composition affects postprandial fatty acid oxidation. *Am J Physiol* 1993; 246 (6 Pt 2):R1065-70.

19. Maughan RJ, Greenhaff PL, Gleeson M, Fenn CE, Leiper JB. The effect of dietary carbohydrate intake on the metabolic response to prolonged walking on consecutive days. *Eur J Appl Physiol* 1987; 56:583-91.

20. Maughan RJ, Williams C, Campbell DM, Hepburn D. Fat and carbohydrate metabolism during low intensity exercise: effects of the availability of muscle glycogen. *Eur J Appl Physiol* 1978; 39:7-16.

21. Felley CP, Felley EM, Van Melle GD, Frascarolo P, Jequier E, Felber JP. Impairment of glucose disposal by infusion of triglycerides in humans: role of glycemia. *Am J Physiol* 1989; 256:E747-E752.

22. Clark KG, Rognstad R, Katz J. Lipogenesis in rat hepatocytes. *J Biol Chem* 1973; 249:2028-36.

23. Salmon DMW, Bowen NL, Hems DA. Synthesis of fatty acids in the perfused mouse liver. *J Biochem* 1974;142:611.

24. Acheson KJ, Flatt JP, Jequier E. Glycogen synthesis versus lipogenesis after a 500 gram carbohydrate meal in man. *Metabolism* 1982; 1234-1240.

25. Acheson KJ, Schutz Y, Bessard T, Ravussin E, Jequier E, Flatt JP. Nutritional influences on lipogenesis and thermogenesis after a carbohydrate meal. *Am J Physiol* 1984; 246:E62-E70.

26. Acheson KJ, Thelin A, Rabussin E, Arnaud MJ, Jequier E. Contribution of 500g naturally labeled ^{13}C dextrin maltose to total carbohydrate utilization and the effect of the antecedent diet in man. *Am J Clin Nutr* 1985; 41:881-90.

27. Hellerstein MK, Christiansen M, Kaempfer S, Kletke C, Wu K, Reid JS, Mulligan K, Hellerstein NS, Shackleton CHL. Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *J Clin Invest* 1991; 87:1841-52.

28. Edholm OG, Fletcher JG, Widdowson EM, McCance RA. The energy expenditure and food intake of individual men. *Br J Nutr* 1955; 9:286-300.

29. Passmore R, Thompson JG, Warnock GM. A balance sheet of the estimation of energy intake and energy expenditures as measured by indirect calorimetry. *Br J Nutr* 1952; 6:253-264.

30. Murray RK, Granner DK, Mayes PA, Rodwell VW. *Harper's Biochemistry*, 23rd ed. Norwalk, CT: Appleton & Lange, 1993.

31. Brody T. *Nutritional Biochemistry* San Diego, CA: Academic Press, Inc., 1994.

32. Eckel RH. Lipoprotein lipase: a multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 1989;320:1060-8.

33. Mahan KL, Arlin M. *Krause's Food, Nutrition & Diet Therapy,* 8th ed. Philadelphia, PA: W.B. Saunders Company, 1992.

34. Kahn CR. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. *Metabolism* 1978; 27(12 Suppl 2):1893-902.

35. Smith U. Carbohydrates, fat, and insulin action. *Am J Clin Nutr* 1994; 59(suppl):686S-9S.

36. Randle PJ, Garland PB, Newsholme EA, Holes CN. The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1963; 1:785-89.

37. Olefsky JM, Reaven GM, Farquhar JW. Effects of weight reduction in obesity: studies of lipid and carbohydrate metabolism in normal and hyperlipoproteinemic subjects. *J Clin Invest* 1974; 53:64-76.

38. Farese RV, Yst TJ, Eckel RH. Tissue-specific regulation of lipoprotein lipase activity by insulin/glucose in normal-weight humans. *Metabolism* 1991; 40:214-6.

39. American Diabetes Association. Nutritional recommendations and principles for individuals with diabetes mellitus: 1986. *Diabetes Care* 1987; 10:126-32.

40. Himsworth HP. The dietetic factor determining the glucose tolerance and sensitivity to insulin of healthy men. *Clin Sci* 1935; 2:67-94.

41. Brunzell JD, Lerner RL, Hazzard WR, Porte D, Bierman EL. Improved glucose tolerance with high carbohydrate feeding in mild diabetes. *N Engl J Med* 1971; 284:521-4.

42. Anderson JW. Effect of carbohydrate restriction and high carbohydrate diets on men with chemical diabetes. *Am J Clin Nutr* 1977; 30:402-8.

43. Kolterman OG, Green field M, Reaven GM, Saekow M, Olefsky JM. Effect of a high carbohydrate diet on insulin binding to adipocytes and on insulin action *in vivo* in man. *Diabetes* 1979; 28:731-6.

44. Groop LC, Salorant C, Shank M, Bonadonna RC, Ferrannini E, DeFronzo RA. The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1991; 72:96-107.

45. Hjollund E, Pedersen O, Richelsen B, Beck-Nielsen H, Sorensen NS. Increased insulin binding to adipocytes and monocytes and increased insulin sensitivity of glucose transport and metabolism in adipocytes from non-insulin-dependent diabetics after a low-fat/high-starch/high-fiber diet. *Metabolism* 1983;32:1067-75.

46. Anderson JW. The role of dietary carbohydrate and fiber in the control of diabetes. *Adv Intern Med* 1980; 26:67-96.

47. Anderson JW, Chen WL. Plant fiber. Carbohydrate and lipid metabolism. *Am J Clin Nutr* 1979; 32:346-63.

48. Villaume C, Beck B, Gariot P, Desalme A, Debry G. Long-term evolution of the effect of bran ingestion on meal-induced glucose and insulin responses in healthy man. *Am J Clin Nutr* 1984; 40:1023-6.

49. Gambera P, Davis P, Schneeman B. Use of the food guide pyramid and dietary guidelines to improve dietary intake and reduce cardiovascular risk in active duty Air Force members. *J Am Diet Assoc* 1995; in press.

50. Kinmouth AL, Mangus RM, Jenkins PA, Smith MA, Baum JD. Whole foods and increased dietary fiber improve blood glucose control in diabetic children. *Arch Dis Child* 1982; 57:187-94.

51. Fukagawa NK. Anderson JW, Hageman G, Young VR, Minaker KL. High-carbohydrate, high-fiber diets increase peripheral insulin sensitivity in healthy and old adults. *Am J Clin Nutr* 1990; 52:524-8.

52. Chen M, Bergman RN, Porte D. Insulin resistance and β-cell dysfunction in aging: the importance of dietary carbohydrate. *J Clin Endocrinol Metab* 1988; 67:951-7.

53. Reaven GM. How high the carbohydrate? *Diabetologia* 1980; 19:409-13.

54. Coulston AM, Liu GC, Reaven GM. Plasma glucose, insulin and lipid responses to high-carbohydrate low-fat diets in normal humans. *Metabolism* 1983; 32(1):52-6.

55. Riccardi G, Rivellese A, Pacioni D, Genovese S, Mastranzo P, Mancini M. Separate influence of dietary carbohydrate and fibre on the metabolic control in diabetes. *Diabetologia* 1984; 26:116-21.

56. Garg A, Bantle JP, Henry RR, Coulston AM, Griver KA, Raatz SK, Brinkley L, Chen I, Grundy SM, Huet BA, Reaven GM. Effects of varying carbohydrate content of diet in patients with non-insulin-dependent diabetes mellitus. *JAMA* 1994;271(18):1421-8.

57. Borkman M, Campbell LV, Chisholm DJ, Storlien LH. Comparison of the effects on insulin sensitivity of high carbohydrate and high fat diets in normal subjects. *J Clin Endocrinol Metab* 1991; 72:432-37.

58. Leclerc I, Davignon I, Lopez D, Garrel D. No change in glucose tolerance and substrate oxidation after a high-carbohydrate, low-fat diet. *Metabolism* 1983; 42(3):365-70.

59. Costill DL. Carbohydrate for athletic training and performance. *Asociacion Medica de Puerto Rico* 1991; 83(8):350-3.

60. Jequier E. Carbohydrates as a source of energy. *Am J Clin Nutr* 1994; 59(suppl):682S-5S.

61. Passmore R, Swindells YE. Observations on the respiratory quotients and weight gain of man after eating large quantities of carbohydrate. *Br J Nutr* 1963; 17:331-9.

62. Schutz Y, Acheson KJ, Jequier E. Twenty-four hour energy expenditure and thermogenesis: response to progressive carbohydrate overfeeding in man. *Int J Obes* 1985; 9(Suppl 2):111-4.

63. Abbott WGH, Howard BV, Christin L, Freymond D, Lilliojo S, Boyce VL, Anderson TE, Bogardus C, Ravussin E. Short-term energy balance: Relationship with protein, carbohydrate and fat balances. *Am J Physiol* 1988; 255:E322-E337.

64. Schutz Y, Flatt JP, Jequier E. Failure of dietary fat to promote fat oxidation: A factor favoring the development of obesity. *Am J Clin Nutr* 1989; 50:307-14.

65. Flatt JP. Opposite effects of variations in food intake on carbohydrate and fat oxidation in ad libitum fed mice. *J Nutr Biochem* 1991; 2:186-92.

66. Flatt JP, Ravussin E, Acheson KJ, Jequier E. Effects of dietary fat on post-prandial substrate oxidation and on carbohydrate and fat balances. *J Clin Invest* 1985; 76:1019-24.

67. Acheson KJ, Schutz Y, Bessard T, Anantharaman K, Flatt JP, Jequier E. Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man. *Am J Clin Nutr* 1988; 48:240-7.

68. Hirsch J. Role and benefits of carbohydrate in the diet: key issues for future dietary guidelines. *Am J Clin Nutr* 1995; 61(4(S):996S-1000S.

69. Hudgins L, Hellerstein M, Seldman C, Diakun J, Hirsch J. Increased de novo lipogenesis on a eucaloric low fat high carbohydrate diet does not alter energy expenditure (Abstract). *Obesity Research* 1993; 1(Suppl 2):92S.

70. Nishina PM, Freedland RA. Effects of propionate on lipid biosynthesis in isolated rat hepatoctes. *J Nutr* 1990; 120:668-73.

71. Cahill GF. Physiology of insulin in man. *Diabetes* 1971; 20:785-99.

72. Golay A, Felber JP, Meyer HU, Curchod B, Maeder E, Jequier E. Study of lipid metabolism in obesity diabetes. *Metabolism* 1984; 33:111-116.

73. Bortz WM, Pavle P, Haff AC, Holmes WL. Glycerol turnover and oxidation in man. *J Clin Invest* 1972; 51:1537-46.

74. Van Amelsvoort JMM, Van Stratum P, Kraal JH, Lussenburg RN, Houtsmuller UMT. Effects of varying the carbohydrate: fat ratio in a hot lunch on postprandial variables in male volunteers. *Br J Nutr* 1989; 61:267-83.

75. Van Amelsvoort JMM, Van Stratum P, Kraal JH, Lussenburg RN, Dubbelman GP. Minor difference in postprandial responses of men between starch and sugar when replacing fat in a normal meal. *Br J Nutr* 1990; 63:37-51.

76. Brooks GA. Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. *Fed Proc* 1986; 45:2924-29.

77. Kreisberg RA. Glucose-lactate inter-relations in man. *N Engl J Med* 1972; 287:132-7.

78. Wasserman K, McIlroy MB. Detecting the threshold of anaerobic metabolism *Am J Cardio* 1964; 14:844-52.

79. Wasserman K, Whipp BJ, Koyal SN, Beaver WL. Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol* 1973; 35:236-43.

80. Brooks GA. Anaerobic threshold: review of the concept and directions for future research. *Med Sci Sports Exercise* 1985; 17:22-31.

81. Mazzeo RS, Brooks GA, Schoeller DA, Budinger TF. Disposal of blood [1-^{13}C] lactate in humans during rest and exercise. *J Appl Physiol* 1986; 60:232-41.

82. Doar JWH, Cramp DG, Maw DSI, Seed M, Wynn V. Blood pyruvate and lactate levels during oral and intravenous glucose tolerance tests in diabetes mellitus. *Clin Sci* 1970; 39:259.

83. Felig P, Wahren J, Hendler R. Influence of oral glucose ingestion on splanchnic glucose and gluconeogenic substrate metabolism in man. *Diabetes* 1975; 24(3):468-75.

84. Brooks GA, Fahey TD. *Exercise Physiology: Human Bioenergetics and Its Applications.* New York: John Wiley and Sons, 1984. 67-95.

85. Issekutz B. Effect of β-adrenergic blockade on lactate turnover in exercising dogs. *J Appl Physiol* 1984.; 57:1754-59.

86. Hems DA, Whitton PD, Taylor EA. Glycogen synthesis in the perfused liver of the starved rat. *Biochem J* 1972; 129:529-38.

87. Geelen MJH. Restoration of glycogenesis in hepatocytes from starved rats. *Life Sci* 1977; 20:1027-34.

88. Boyd ME, Albright EB, Foster DW, McGarry JD. In vitro reversal of the fasting state of liver metabolism in the rat. Reevaluation of the roles of insulin and glucose. *J Clin Invest* 1981; 68:142-52.

89. Newgard CB, Hirsch LF, Foster DW, McGarry JD. Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. *J Biol Chem* 1983; 258:8046-52.

90. Radziuk J. The liver and glycogen metabolism. *J Parenteral Enteral Nutr* 1991; 15:77S-81S.

91. Radziuk J. Glycogen in humans. II. Gluconeogenic formation after oral and intravenous glucose. *Am J Physiol* 1989; 257:E158-69.

92. Magnusson I, Chandramouli V, Schumann WC, Kumaran K, Wahren J, Landau BR. Pathways of hepatic glycogen formation in humans following the ingestion of a glucose load in the fed state. *Metabolism* 1989; 38:583-85.

93. Katz J, McGarry JD. The glucose paradox: is glucose a substrate for liver metabolism? *J Clin Invest* 1984; 74:1901-9.

94. Foster DW. From glycogen and ketones--and back. *Diabetes* 1984; 33:1188-99.

95. McGarry DJ, Woeltje KF, Kuwajima M, Foster DW. Regulation of ketogenesis and the renaissance of carnitine palmitoyl transferase. *Diabetes/Metabolism Reviews* 1989; 5(3):271-84.

96. Ontko JA. Metabolism of free fatty acids in isolated lever cells. Factors affecting the partition between esterification and oxidation. *J Biol Chem* 1972; 247:1788-1800.

97. Woodside WF, Heimberg M. Hepatic metabolism of free fatty acids in experimental diabetes. *Isr J Med Sci* 1972; 8:309-16.

98. McGarry JD, Foster DW. Regulation of hepatic fatty acid oxidation and ketone body production. *Ann Rev Biochem* 1980; 49:395-420.

99. Garlick PJ. Evaluation of the formulae for calculating nutrient disposal rates from respiratory gas measurements in fed subjects. *Hum Nutr:Clin Nutr* 1987; 41C:165-76.

100. Neill GM, Bruce AC, Ralph A, James WPT. Inter-individual differences in fasting nutrient oxidation and the influence of diet composition. *Int J Obes* 1988; 12:455-63.

101. Ferrannini E. The theoretical basis of indirect calorimetry: a review. *Metabolism* 1988; 37:287-301.

102. Flatt JP. Dietary fat, carbohydrate balance, and weight maintenance: effects of exercise. *Am J Clin Nutr* 1987; 45:296-306.

103. Schutz Y. The adjustment of energy expenditure and oxidation to energy intake: the role of carbohydrate and fat balance. *Int J Obes* 1993; 17(3):23S-7S.

104. Nuutila P, Kolvisto VA, Knuuti J, Ruotsalainen U, Teras M, Haaparanta M, Bergman J, Solin O, Voipio-Pulkki LM, Wegelius U, Ykio-Jarvinen H. Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. *J Cin Invest* 1992; 89:1767-74.

105. Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA. Effect of fatty acids on glucose production and utilization in man. *J Clin Invest* 1983; 72:1737-47.

106. Thiebaud D, DeFronzo RA, Jacot E, Golay A, Acheson K, Maeder E, Jequier E, Felber JP. Effect of long chain triglyceride infusion on glucose metabolism in man. *Metabolism* 1982; 31(11):1128-36.

107. Thiebaud D, Jacot E, DeFronzo RA, Maeder E, Jequier E, Felber JP. The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes* 1982; 31:957-63.

108. Nurjhan N, Campbell P, Kennedy FP, Miles JM, Gerich JE. Insulin dose-response characteristics for suppression of glycerol release and conversion to glucose in humans. *Diabetes* 1986; 35:1326-31.

109. Campbell PJ, Carlson MG, Hill JO, Nurjhan N. Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. *Am J Physiol* 1992; 263:E1063-E1069.

110. Flatt JP. Role of the increased adipose tissue mass in the apparent insulin insensitivity of obesity. *Am J Clin Nutr* 1972; 1189-92.

111. Golay A, Swislocki ALM, Chen Y-D I, Jaspan JB, Reaven GM. Effect of obesity on ambient plasma glucose, free fatty acids, insulin, growth hormone, and glucagon concentrations. *J Clin Endocrinol Metab* 1986; 63:481-4.

112. Groop LC, Bonadonna RC, Simonson DC, Petrides AS, Shank M, DeFronzo RA. Effect of insulin on oxidative and nonoxidative pathways of free fatty acid metabolism in human obesity. *Am J Physiol* 1992; 263:E79-E84.

113. Gomez F, Jequier E, Chabot V, Buber V, Felber JP. Carbohydrate and lipid oxidation in normal human subjects: its influence on glucose tolerance and insulin response to glucose. *Metabolism* 1972; 21(5):381-91.

114. Felber JP, Vannotti A. Effects of fat infusion on glucose tolerance and insulin plasma levels. *Med Exp* 1964; 10:153-6.

115. Schalch DS, Kipnis DM. Abnormalities in carbohydrate tolerance associated with elevated plasma nonesterified fatty acids. *J Clin Invest* 1965; 44:2010-20.

116. Felber JP, Ferrannini E, Golay A, Meyer HU, Theibaud D, Curchod B, Maeder E, Jequier E, DeFronzo RA. Role of lipid oxidation in pathogenesis of insulin resistance of obesity and type II diabetes. D*iabetes* 1987; 36:1341-50.

117. Boden G, Ray TK, Smith RH, Owen OE. Carbohydrate oxidation and storage in non-insulin-dependent diabetic patients: effects of improving glycemic control. *Diabetes* 1983; 32:982-7.

118. Bogardus C, Lillioja S, Howard BV, Reaven G, Mott D. Relationship between insulin secretion, insulin action, and fasting plasma glucose concentration in nondiabetic and noninsulin-dependent diabetic subjects. *J Clin Invest* 1984; 74:1238-46.

119. Rousselle J, Buckert A, Pahud P, Jequier E, Felber JP. Relationship between glucose oxidation and glucose tolerance in man. *Metabolism* 1982; 31:866-70.

120. Felber JP, Kleiber H, Jallut D, Golay A, Frascarolo P, Jequier E. Effect of lipid oxidation on the regulation of glucose uptake and glucose storage in man (Abstract). *Diabetes* 1988; 37 (Suppl 1):220A.

121. Connor WE, Connor SL. Importance of diet in the treatment of familial hypercholesterolemia. *Am J Cardio* 1993; 72(10):42D-53D.

122. Schafer EF, Levy RI, Ernst ND, Van Sant DV, Brewer HB. The effects of low cholesterol, high polyunsaturated fat, and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholesterolemic subjects. *Am J Clin Nutr* 1981; 34:1758-63.

123. Brinton EA, Eisenberg S, Breslow JL. A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. *J Clin Invest* 1990; 85:144-51.

124. Thuesen L, Henriksen LB, Diet C, Engby B. One-year experience with a low-fat, low-cholesterol diet in patients with coronary heart disease. *Am J Clin Nutr* 1986; 44:212-9.

125. Kasim SE, Martino S, Kim P-N, Khilnani S, Boomer A, Depper J, Reading BA, Heilbrun LK. Dietary and anthropometric determinants of plasma lipoproteins during a long-term low-fat diet in healthy women. *Am J Clin Nutr* 1993; 57:146-53.

126. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. *Lancet* 1987; 1(8525):122-5.

127. Rivellese AA, Auletta P, Marotta G, Saldalamacchia G, Giacco A, Mastrilli VA, Vaccaro O, Riccardi G. Long term metabolic effects of two dietary methods of treating hyperlipidaemia. *Bmj* 1994; 308:227-31.

128. Hegstad DM, McGandy RB, Myers ML, Stare FJ. Quantitative effect of dietary fat on serum cholesterol in man. *Am J Clin Nutr* 1965; 17:281-95.

129. Geil PB, Anderson JW, Gustafson NJ. Women and men with hypercholesterolemia respond similarly to an American Heart Association Step 1 diet. *J Am Diet Assoc* 1995; 95:436-41.

130. Kris-Etherton PM, Krummel D. Role of nutrition in the prevention and treatment of coronary heart disease in women. *J Am Diet Assoc* 1993; 93:987-93.

131. Cominacini L, Zocca I, Garbin U, Davoli A, Compri R, Brunetti L, Bosello O. Long-term effect of a low-fat, high-carbohydrate diet on plasma lipids of patients affected by familial endogenous hypertriglyceridemia. *Am J Clin Nutr* 1988; 48:57-65.

132. Kris-Etherton PM, Krummel D, Russell ME, Dreon D, Mackey S, Borchers J, Wood PD. The effect of diet on plasma lipids lipoproteins, and coronary heart disease. *J Am Diet Assoc* 1988; 88(11):1373-400.

133. Austin MA. Plasma triglyceride as a risk factor for coronary heart disease. *Am J of Epidem* 1989; 129(2):249-59.

134. Mancini M, Mattock M, Rabaya E, Chait A, Lewis B. Studies of the mechanisms of carbohydrate-induced lipaemia in normal man. *Atherosclerosis* 1973; 17:445-54.

135. Farquhar JW, Frank A, Gross RC, Reaven GM. Glucose, insulin, and triglyceride responses to high and low carbohydrate diets in man. *J Clin Invest* 1966; 45(10):1648-56.

136. Ullmann D, Connor WE, Hatcher LF, Connor SL, Favell DP. Will a high-carbohydrate, low-fat diet lower plasma lipids and lipoproteins without producing hypertriglyceridemia? *Arteriosclerosis and Thrombosis* 1991; 11:1059-67.

137. Truswell SA. Food carbohydrates and plasma lipids--an update. *Am J Clin Nutr* 1994; 59(suppl):710S-8S.

138. Anderson JW, Chen W-J, Sieling B. Hypolipidemic effects of high-carbohydrate, high-fiber diets. *Metabolism* 1980; 29(6):551-7.

139. Legato MJ. Cardiovascular disease in women: what's different? what's new? what's unresolved? *Ann New York Acad Sci* 1994; 736:147-57.

140. Castelli WP, Doyle JT, Gordon T, et al. HDL cholesterol and other lipids in coronary heart disease: the Cooperative Lipoprotein Phenotyping Study. *Circulation* 1977; 55:767-72.

141. Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial Results. I. Reduction in incidence of coronary heart disease. *JAMA*. 1984; 251:351-64.

142. Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial Results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA* 1984; 251:365-74.

143. NIH Consensus Development Panel on Triglyceride, High-Density Lipoprotein, and Coronary Heart Disease. Triglyceride, high-density lipoprotein, and coronary heart disease. *JAMA* 1993; 269(4):505-10.

144. Human subjects Protocol No.: 94518R. HRSC, Approved: 3/2/94.

145. National Research Council. *Recommended Dietary Allowances*. 10th ed. National Academy Press, Washington, D.C. 1989.

146. Allain CA, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20:470-5.

147. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973; 19:476-82.

148. Warnick GR, Benderson J, Albers JJ: Dextran sulfate precipitation procedure for quantitation of high-density lipoprotein. *Clin Chem* 1982; 28:1379-88.
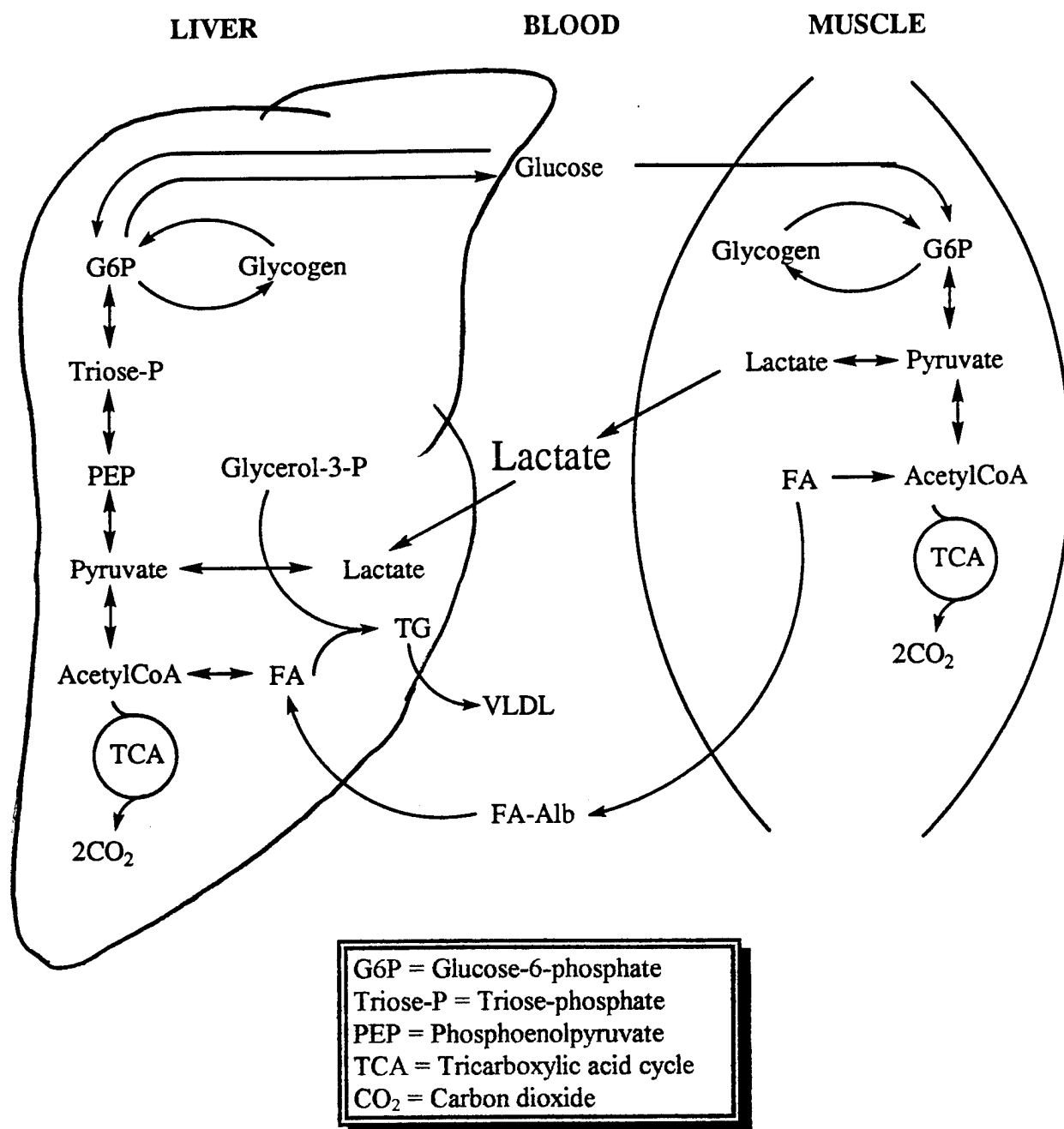
149. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18:499-502.

150. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969; 6:24.

151. Barhan D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxdase system. *Analyst 97* 1972; 97:142-5

152. Boobis LH, Maughan RJ. A simple one-step enzymatic fluorometric method for the determination of glycerol in 20 *ul* of plasma. *Clinica Chimica Acta* 1983; 132:173-9.

153. Sheppard L, Kristal AR, Kushi LH. Weight loss in women participating in a randomized trial of low-fat diets. *Am J Clin Nutr* 1992; 55:934-42.

154. Thomas CD, Peters JC, Reed GW, Abumrad NN, Sun M, Hill JO. Nutrient balance and energy expenditure during ad libitum feeding of high-fat and high-carbohydrate diets in humans. *Am J Clin Nutr* 1992; 55:934-42.

155. Boozer CN, Brasseur A, Atkinson RL. Dietary fat affects weight loss and adiposity during energy restriction in rats. *Am J Clin Nutr* 1993; 58:846-52.

156. Rolls BJ. Carbohydrates, fats, and satiety. *Am J Clin Nutr* 1995; 61(suppl):960S-7S.

157. Olefsky JM. Insulin resistance and insulin action. *Diabetes* 1981; 30:148-62.

158. Laakso M. How good a marker are insulin levels for insulin resistance? *Am J Epidemiol* 1993; 137:959-65.

159. Wako Pure Chemical Industries, Ltd., Richmond VA, NEFA C method, Code No. 990-75401.

160. Reaven GM, Lerner RL, Stern MP, Farquhar JW. Role of insulin in endogenous hypertriglyceridemia. *J Clin Invest* 1967; 46(11):1756-67.

161. Sheu WH, Shieh SM, Fuh MMT, Shen DDC, Jeng SCY, Chen YDI, Reaven GM. Insulin resistance, glucose intolerance, and hyperinsulinemia. *Arteriosclerosis and Thrombosis* 1993; 13:367-70.

162. Goldstein DJ. Beneficial health effects of modest weight loss. *Int J Obesity* 1992; 16:397-416.

163. Webber J, Donaldson M, Allison SP, Fukagawa NK, Macdonald IA. The effects of weight loss in obese subjects on the thermogenic, metabolic, and haemodynamic responses to the glucose clamp. *Int J Obesity* 1994; 18:725-30.

164. Franssila-Krallunki A, Rissanen A, Ekstrand A, Ollus A, Groop L. Effects of weight loss on substrate oxidation, energy expenditure, and insulin sensitivity in obese individuals. *Am J Clin Nutr* 1992; 55:356-61.

165. Laurell S, Tibbling G. An enzymatic fluorometric micromethod for the determination of glycerol. *Clin Chim Acta* 1966; 13:317-22.

166. Ferrannini E, Natali A, Brandi LS, BonadonnaR, DeKreutzemberg SV, DelPrato S, Santoro D. Metabolic and thermogenic effects of lactate infusion in humans. *Am J Phys* 1993; 265:E504-512.

167. Tomita Y, Abraham S, Noda C, Ichihara A. Pyruvate stimulates hormonal induction of lipogenic enzymes in primary cultured rat hepatocytes. *Biochimica et Biophysica Acta* 1993; 1170:253-7.

168. Williamson DH, Mellanby J, Krebs HA. Enzymatic determination of D[-]β-hydroxybutyric acid and acetoacetic acid in blood. *Biochem J* 1962; 82:90.

169. Barnard RF. Effects of life-style modification on serum lipids. *Arch Intern Med* 1991; 151:1389-94.

170. Hunninghake DB, Stein EA, Dujovne CA, Harris WS, Feldman EB, Miller VT, Tobert JA, Laskarzewski PM, Quitter E, Held J, Taylor AM, Hopper S, Leonard S, Brewer BK. The efficacy of intensive dietary therapy alone or combined with Lovastatin in outpatients with hypercholesterolemia. *N Eng J Med* 1993; 328:1213-9.

171. Kannel WB. Metabolic risk factors for coronary heart disease in women: perspective from the Framingham Study. *Am Heart J* 1987; 114:413-9.

172. Jacobs DR, Mebane IL, Bangdiwala SI, Criqui MH, Tyroler HA. High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: the Follow-up Study of the Lipid Research Clinics' Prevalence Study. *Am J Epidemiol* 1990; 131:32-47.

173. Brunner D, Weisbort J, Meshulam N, et al. Relation of serum total cholesterol and high-density lipoprotein cholesterol percentage to the incidence of definite coronary events: twenty-year follow-up of the Donolo-Tel Aviv Prospective Coronary Artery Disease Study. *Am J Cardiol* 1987; 59:1271-6.

174. Kinosian B, Glick H, Garland G. Cholesterol and coronary heart disease: predicting risks by levels and ratios. *Ann Intern Med* 1994; 121:641-7.

175. Laws A, King AC, Haskell WL, Reaven GM. Metabolic and behavioral covariates of high density lipoprotein-cholesterol and triglyceride concentrations in postmenopausal women. *J Am Geriatr Soc* 1993; 41:1289-94.

176. Committee to Develop Criteria for Evaluating the Outcomes of Approaches to Prevent and Treat Obesity. Food and Nutrition Board. *Weighing the options: criteria for evaluating weight-management programs.* Washington, D.C.: National Academy Press, 1995.

177. Azain MJ, Martin RF. Hepatic palmitate metabolism in fasting Zucker rats-- Effects of lactate on partitioning. *Amer J Physiol* 1984; 246(6 Pt 2):R1011-4.

APPENDIX A

Metabolic Pathways

LIVER   BLOOD   MUSCLE

G6P = Glucose-6-phosphate
Triose-P = Triose-phosphate
PEP = Phosphoenolpyruvate
TCA = Tricarboxylic acid cycle
CO_2 = Carbon dioxide

APPENDIX B

Personal Data Survey

Personal Data Survey

All information on this questionnaire is completely confidential, and will only be used to help us assist you and the study better. Please fill in the following lines with the information asked. If you have any questions please feel free to ask.

Name: _____

Address: _____

_____

Telephone Number: _____

Age _____   Race (Ethnicity) _____

Are you interested in participating in this study? _____

     If **yes** please skip down to PART B.

     If **no** could you please tell us what made you decide not to participate in this study?

_____

_____

How might we improve or change the study to make it more appealing? _____

_____

PART B

Please circle the correct answer or where indicated fill in the blank. Please be as honest and accurate as you can. Do not hesitate to ask questions.

    1. When was your last menstrual cycle? _____

    2. Do you take any doctor prescribed medications? yes / no

       If yes, Please list any medications you are presently taking?

       _____

       _____

       _____

3. Are you taking estrogen? yes / no

    If yes are you taking it continuously (daily) or cycled? _____

4. Do you have diabetes mellitus? yes / no

5. Do you have kidney disease or renal failure? yes / no

6. Do you have liver disease or failure? yes / no

7. Do you have known cancer? yes / no

    If yes, please describe type and any treatments?

    _____

    _____

8. Do you know your blood cholesterol level? _____

9. Please list any other major illnesses or diseases you

    have? _____

    _____

    _____

10. Do you smoke cigarettes? yes / no

    If yes how much/often? _____

11. Do you use any other tobacco product (examples pipe, chewing tobacco, ...)?
    yes / no

    If yes, what type and how often? _____

12. How often do you drink alcoholic beverages?   daily /

    2-3x/week / 4-5x/week / weekly / 2-3x/month /
    monthly / less than once a month / never

13. Who do you currently live with? _____

    _____

14. How many meals per day do you eat at home? _____

15. How many times per day do you eat? ___ meals___ snacks

16. Who else eats meals with you at home? _____

17. Who prepares the meals you eat? _____

18. Do you prepare meals for anyone else other than
    yourself? yes / no

    If yes, who else do you prepare meals for? _____

    _____

    How often do you prepare meals for others? _____

19. How often do you eat out? _____

20. Do you work? yes / no

    If yes, where do you work? _____

    And what hours and days do you work? _____

21. What source of transportation do you have (for example do you use the bus/ drive
    yourself/ a family member...)? _____

22. Do you consider yourself to have reliable
    transportation? yes / no

23. Would you have a problem coming to the UC Davis Medical Center on Stockton
    Boulevard **every** evening Monday through Friday for four months to eat dinner
    and receive the rest of your meals at no charge for this time period? yes / no

24. Do you have any known food allergies? yes / no

    If yes, what foods are you allergic to? _____

    _____

25. Please list any foods you absolutely will not eat?

    _____

    _____

26. Do you have any ethnic/ religious/ or other food practices (for example days of fasting, you can not eat pork or beef, or can only eat certain foods on certain holidays)? yes / no

   If yes, please list and explain? _____

   _____

27. Do you drink milk? yes / no

   If yes, what type of milk do you drink? _____

28. If asked to drink whole milk for the study would you? Yes / No

29. If asked to drink nonfat milk for the study would you? Yes / No

30. When do you have plans to go on vacation away from Sacramento? Yes / No

   If yes, please list the dates which you plan to be away over the next year?

   _____

   _____

31. Can you commit to being available for four months in a row for this study? Yes / No

32. What problems do you anticipate encountering during this study?

   _____

   _____

33. Please look over the menu for this study. Are there any foods on this menu which you can not or will not eat? Yes / No

   If yes, please list and explain: _____

   _____

   _____

APPENDIX C

Human Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

UNIVERSITY OF CALIFORNIA, DAVIS

Title of Study: High Density Lipoproteins, Biomarkers of Low-Fat Diet

Investigator's Name: Sidika E. Kasim, M.D.

Department: Internal Medicine/Division of Clinical Nutrition and Metabolism

Phone: (916) 752-6778

PURPOSE:

You are being asked to participate in a research study. We hope to find some objective and unbiased measurement which can reflect the amount of the fat intake in the diet. It is known that frequency of certain types of cancers are lower in the populations who consume low-fat diets. However research studies produce conflicting results about the relationships between dietary fat and cancer. Part of the problem is that assessment of the dietary fat entirely relies on the verbal information from the study participants. Reliable measurement of dietary fat intake is not yet available.

PROCEDURES:

Diet: If you decide to volunteer, we will ask you to participate in the study for one year. During the first four months of the study you will consume only the food provided by us. During the first month this diet will be similar to your own diet. During the remaining three months fat content of the diet will be considerably less (25%-fat for two months and 15%-fat for one month). Monday through Friday, you will eat dinner at the University of California Davis Medical Center located on 2315 Stockton Boulevard in Sacramento. The breakfasts, lunches and snacks will be given as take-out meals. On Saturdays and Sundays all the meals will be provided as take-outs.

After this period you will follow a fat-restricted diet (15%) on your own. We will teach you how to follow a low fat diet during a series of ten evening classes. You will learn how to shop, prepare and cook low-fat food. During the following two months you will be asked to come to a pot-luck dinner once a week, where we will all share recipes and discuss the various issues which may come up. After the first two months, the pot-luck dinners will be held every other week.

_____
Participant's Initials

Blood Drawing: During the first four months, you will have blood drawn every other week, for nine times. Four tubes of blood (approximately one ounces) will be collected each time. The blood samples will be obtained before the breakfast, after a 14-hour overnight fast. During the remaining eight months of the study, you will have the same amounts of blood drawn once every other month (total of four draws).

Other Tests: You will be weighed every day. Before each blood draw, we will measure your weight and blood pressure. Five times during the one year period, we will measure your waist and hip circumferences and the resting energy expenditure. Measurement of resting energy expenditure sitting quietly in a comfortable chair for thirty minutes and breathing room air into a transparent hood placed on your head and shoulders. We will also measure your body fat composition by a method called electrical impedance. This method involves lying quietly on a bed for five minutes, while a low voltage electricity (as strong as a flash light battery) goes through your body. There is no perception of electricity or pain during this procedure. Finally, three times during the one year period we will obtain a very small piece of a fat tissue by aspiration with a needle from your hip, after numbing the area.

In this trial we are offering you a substudy of your blood lipid (fat) responses after consumption of a typical meal (ie. your post-prandial response). Recent research has suggested that the post-prandial levels of your blood fat may be affected by diet, which may in turn be associated with risk of various diseases. It is important that you understand that participation in this substudy is voluntary and that you may participate in the trial without volunteering for the substudy.

If you decide to participate in the post-prandial substudy you will be asked to spend four nonconsecutive eight hour days at the research facility during the first four month period of the trail (approximately one test per month). On each occasion, you will be asked to fast overnight before coming in to have an intravenous line placed in your forearm and your blood drawn. You will then be asked to consume a test meal of typically consumed foods. After this meal you will be requested not to eat or drink for the remaining six hours. Through the intravenous line blood will be drawn 3 times during the first hour, and then every hour for the remaining five hours. The total amount of blood drawn will be five and a half ounces, which is equivalent to approximately one-third of a regular blood donation to the Red Cross.

During the day, you are free to move around the facility and we will be providing some entertainment in the form of movies, reading material, and board games/cards.

(   ) **I wish to participate in the substudy**

(   ) **I do not wish to participate in the substudy**

_____

Participant's Initials

RISKS:

Diet: To follow a low fat diet for a year requires a major commitment and changes in life style. Especially the first four months of the diet which will require the consumption of all meals at a designated site may be an inconvenience.

Blood Drawing: Discomfort of blood drawing, small chance of bleeding and infection.

Adipose Tissue Aspiration: Discomfort of needle insertion, small chance of bleeding and infection.

BENEFITS:

Food will be free of charge during the first three months. All the tests will also be free and you will be informed about the results. You will receive an excellent education about low-fat dieting.

CONFIDENTIALITY:

Only the study and laboratory personnel will have an access to the information. Your identity will not be revealed in the publications. However, absolute confidentiality cannot be guaranteed since research documents are not protected from subpoena.

COST/COMPENSATION:

If you are physically injured as a direct result of procedures not done primarily for your own benefit, undertaken at University of California facilities, you will receive medical treatment at no cost. The University of California does not provide any other form of compensation for injury.

_____
Participant's Initials

RIGHT TO REFUSE OR WITHDRAW:
You may change your mind about being in the study and quit after the study has started. The investigator (Dr. Kasim) also may withdraw you from the study at her own discretion.

QUESTIONS:
If you have any questions, please ask us. If you have additional questions later, Dr. Kasim will answer them at 752-6778 (U.C. Davis, Division of Clinical Nutrition and Metabolism Office, TB-156, Davis, CA 95616). She can be reached at night through a pager (dial 762-6603 and after you hear the beep, dial your own phone number).

You will be given a signed and dated copy of this form to keep. You will also be given a copy of the Experimental Subject's Bill of Rights.

****

**YOUR SIGNATURE, BELOW, WILL INDICATE THAT YOU HAVE DECIDED
TO VOLUNTEER AS A RESEARCH SUBJECT
AND THAT YOU HAVE READ AND UNDERSTOOD THE INFORMATION
PROVIDED ABOVE, AND THE BILL OF RIGHTS.**

_____	_____
Date	Signature of participant or legal representative

_____	_____
Date	Signature of participant or legal representative

SEK:dh

UNIVERSITY OF CALIFORNIA, DAVIS

EXPERIMENTAL SUBJECTS' BILL OF RIGHTS

The rights below are the rights of every person who is asked to be in a medical research study. As an experimental subject, you have the following rights:

1) To be told what the study is trying to find out;

2) To be told what will happen to you and whether any of the procedures, drugs, or devices is different from what would be used in standard practice;

3) To be told about the frequent and/or important risks, side effects or discomforts of the things that will happen to you for research purposes;

4) To be told if you can expect any benefit from participating and, if so, what the benefit might be;

5) To be told other choices you have and how they may be better or worse than being in the study;

6) To be allowed to ask any questions concerning the study, both before agreeing to be involved and during the course of the study;

7) To be told what sort of medical treatment is available if any complications arise;

8) To refuse to participate at all or to change you mid about participating after the study is started. This decision will not affect your right to receive the care you would receive if you were not in the study;

9) To receive a copy of the signed and dated consent form;

10) To be free of pressure when considering whether you wish to agree to be in the study.

If you have other questions, please ask the researcher or research assistant. In addition, you may contact the Human Subjects Review Committee, which are concerned with protecting volunteers in research projects. You may reach the committee office by calling (916) 752-2075, from 8:00 am to 5:00 pm, Monday through Friday, or by writing to the Human Subjects Review Committee, Office of Research, 410 Mrak Hall, University of California, Davis, California 95616.

Participant's Initials/Date

APPENDIX D

7-Day Cycle Menus Formulated to Provide:

Cycle I: 35% dietary fat

Cycle II: 25% dietary fat

Cycle III: 15% dietary fat

FAT35.XLS

Cycle 1 Menu

	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
Breakfast	Orange Juice Corn Flakes Banana Muffin Milk Margarine	Applesauce – English Muffin Margarine Milk Rice Krispies	Bagel Cream Cheese Milk Special K Cereal	Cantaloup Blueberry Muffin Oatmeal Milk	Orange Juice Waffles Margarine Syrup	Banana Bran Flakes Milk	Fruited Yogurt Bagel Cream Cheese
Lunch	Baked Manicotti Grapes Bread Margarine	Cottage Cheese Fresh Fruit Muffin Chicken Broth	Tropical Chicken Salad Apple Crisp	Turkey and Cheese Sandwich Green Salad Italian Dressing	Mandarin Rice Salad Bread Margarine	Cranberry Juice Trio Salad Plate Crackers	Pasta Salad Melon Crackers
Dinner	Teriyaki Chicken Rice Baby Carrots	Swedish Turkey Meatballs over Pasta Mixed Vegetables Coffee/Tea/Diet Soda	Roast Beef Baked Potato Margarine Sour Cream Wax Beans Coffee/Tea/Diet Soda	Chicken Lo Mein Broccoli Spears Baked Apple Coffee/Tea/Diet Soda	Roasted Turkey Seasoned Pasta Carrot Coins Pudding Coffee/Tea/Diet Soda	Salisbury Steak Mashed Potatoes Beef Gravy Mixed Vegetables Coffee/Tea/Diet Soda	Carne Guisada Beef Rice French Green Beans
Evening Snack	Sugar Cookie	Ice Cream (for some MUFFIN)	Sugar Cookies (for some MUFFIN)	Chocolate Chip Cookies (for some MUFFIN)	Carmel Crunch Bar (for some MUFFIN)	Gingerbread (for some MUFFIN)	Brownie Milk (for some MUFFIN)

MENU CYCLE 2

FAT25.XLS

	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
Breakfast	Waffles Syrup Margarine Apple	Applesauce English Muffin Margarine Rice Krispies Milk - Nonfat Jelly	Special K Cereal Bagel Milk - Nonfat Cream Cheese Orange Juice	Cantaloup Blueberry Muffin Oatmeal Raisins Milk - Nonfat	Corn Flakes Banana Muffin Milk - Lowfat	Banana Bran Flakes Toast Jelly Milk - Nonfat	Cranberry Juice - 2 Fruited Yogurt Bagel Cream Cheese
Lunch	Baked Manicotti Grapes French Bread Margarine Grape Juice	Cottage Cheese Fresh Fruit Bran Muffin Chicken Broth	Tropical Chicken Salad Apple Crisp Crackers	Turkey Sandwich Green Salad Italian Dressing	Butterscotch Pudding Mandarin Rice Salad Bread Margarine	Apple Juice Trio Salad Plate Crackers	Pasta Salad Bread Jelly Margarine Melon
Dinner	Teriyaki Chicken Rice Carrots	Swedish Turkey Meatballs over Pasta Mixed Vegetables Coffee/Tea/Diet Soda	Roast Beef Baked Potato Margarine Sour Cream Wax Beans Coffee/Tea/Diet Soda	Chicken Lo Mein Broccoli Spears Baked Apple Coffee/Tea/Diet Soda	Roasted Turkey Seasoned Pasta Carrot Coins Coffee/Tea/Diet Soda	Salisbury Steak Mashed Potatoes Beef Gravy Mixed Vegetables Coffee/Tea/Diet Soda	Carne Guisada Beef Rice French Green Beans Coffee/Tea/Diet Soda
Evening Snack	Sugar Cookies Milk - Nonfat cookie(s)	Frozen Yogurt Cranberry Juice muffin(s)	Vanilla Wafers muffin(s)	Chocolate Chip Cookie muffin(s)	Carmel Crunch Bar muffin(s)	Gingerbread Pear muffin(s)	Brownie Milk - Nonfat muffin(s)

FAT15.XLS

MENU CYCLE 3

	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
Breakfast	Waffles Syrup Margarine Apple	Applesauce English Muffin Margarine Rice Krispies Milk – Nonfat	Special K Cereal Bagel Milk – Nonfat Cream Cheese Orange Juice	Cantaloup English Muffin Margarine Oatmeal Raisins Milk – nonfat	Corn Flakes Banana Muffin Peaches Milk – nonfat	Banana Bran Flakes Wheat Toast Jelly Milk – Nonfat	Apple Juice 2 Fruited Yogurt Bagel Cream Cheese
Lunch	Baked Manicotti Grapes French Bread Margarine Grape Juice – 2	Cottage Cheese Fresh Fruit Bran Muffin Chicken Broth Saltine Crackers	Tropical Chicken Salad Apple Crisp Crackers	Turkey Sandwich Green Salad Italian Dressing Orange Angel Food Cake w/ strawberries	Butterscotch Pudding Mandarin Rice Salad Bread Butter	Cranberry Juice Trio Salad Plate Crackers	Pasta Salad Bread Jelly Melon
Dinner	Teriyaki Chicken Rice Carrots	Swedish Turkey Meatballs over Pasta Mixed Vegetables Coffee/Tea/Diet Soda	Roast Beef Baked Potato Margarine Sour Cream Wax Beans Coffee/Tea/Diet Soda	Chicken Lo Mein Broccoli Spears Baked Apple Coffee/Tea/Diet Soda	Roasted Turkey Seasoned Pasta Corn Coffee/Tea/Diet Soda	Salisbury Steak Mashed Potatoes Beef Gravy Mixed Vegetables Coffee/Tea/Diet Soda	Carne Guisada Beef Rice French Green Beans Coffee/Tea/Diet Soda
Evening Snack	Graham Crackers Milk – Nonfat	Frozen Yogurt Cranberry Juice	Angel Food Cake with Cherry topping	Graham Crackers Apple Juice – 2	Vanilla Wafers	Gingersnap Cookies Pear	Brownie Milk – Nonfat

APPENDIX E

List of Free Foods

and

Foods Allowed One Time Per Week

List of Free Foods

These are the only foods allowed outside of what we already provide to you. They are free, meaning allowed in unlimited amount (unless an amount is specified).

Drinks:

Water
Diet sodas (Sugar-free, carbonated beverages)
Unsweetened carbonated water
Club soda
Coffee / Tea (with nothing added)
Sugar free drink mixes (examples include Crystal Light, Sugar-Free Koolaid)
Sugar free tonic water

Fruits:

Cranberries, unsweetened (1/2 cup)
Rhubarb, unsweetened (1/2 cup)

Vegetables:

Cabbage	Celery	Chines Cabbage
Cucumbers	Green Onion	Endive
Escarole	Lettuce	Romaine
Mushrooms	Radishes	Spinach
Zucchini	Onions	

Condiments:

Catsup, Mustard, Horseradish, Lemon juice, Vinegar, Herbs/spices (such as salt, pepper, cinnamon, nutmeg, garlic powder ...)
Fat free salad dressings (2 Tbsp.) - for example Kraft Free
Dill, unsweetened pickles
Sugar Substitutes (such as Equal, Sweet n' Low, saccharin Nutrasweet, aspartame)
Sugar Free Jelly/Jam (1 Tbsp.)
Fat Free Broth - for example Healthy Choice, Pritikin

Sweets:

Sugar-free hard candy (such as Estees)
Sugar-free gelatin (Sugar Free Jell-O brand gelatin)
Sugar-free gum (such as Trident, Care-Free Sugarless gums)

<u>FOODS ALLOWED ONE TIME PER WEEK - THESE ARE NOT ADDITIVE</u> <u>(meaning
if you don't have an item one week you can NOT have two next
week)</u> A week is from Tuesday to the following Tuesday.

ONE TIME DURING THE WEEK YOU MAY HAVE **ONE** OF THE FOLLOWING ITEMS:

1) <u>ALCOHOL</u>:   4 ounces of wine (1/2 cup)
          or 8 ounces of beer (1 cup)
          or 1 ounce of hard liquor (one shot glass)

    OR

2) <u>FRUIT</u>:   1/2 Cup fresh fruit or unsweetened canned fruit
          or 1 small piece of fresh fruit

    OR

3) <u>STARCH</u>:   5 FAT FREE Crackers (such as Nabisco Premium
              Saltines, SnackWells Wheat or Cracked Pepper)
          or 1 FAT FREE Cookie (such as SnackWells or Nabisco
              Fat Free Newtons)

4) <u>VEGETABLE</u>: 1 CUP RAW Vegetables (such as carrots, tomatoes)
          or 1/2 CUP COOKED Vegetables (such as green beans,
              carrots, broccoli, cabbage, cauliflower)

Please remember to record ANY item eaten (include item name,
amount and brand) and give to Wendy Mueller - if you have
questions please call Wendy at 734-0570 or page her at 762-8998.